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THE BOTANICAL GAZETTE

EDITOR: JOHN M. COULTER

APRIL 1923

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A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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THE
BOTANICAL GAZETTE

April 1923

HYPOTHESIS OF FORMATIVE STUFFS AS APPLIED TO BRYOPHYLLUM CALYCINUM[1]

ERNEST REED

(WITH TEN FIGURES)

Introduction

The obscure problems of organ differentiation in plants and animals have prompted considerable effort toward the characterization and identification of a specific substance, or specific substances, fundamentally responsible for the development of the different organs. Several investigators have tried to determine the rules governing the production and movement of these hypothetical substances, and to correlate their movements in the organism with organ development. In the literature of the subject these substances have been known by the very descriptive title of *formative stuffs* or *specific substances*.

It is obvious from the developmental history of any of the higher plants that the several distinctly different organs of each individual are all derived from cells of one kind, and, in the last analysis, from one cell. The problem is, does the individual possess different substances, each of which is responsible for one kind of organ of the plant? Is there a specific substance, or formative stuff, whose sole function is the formation of roots, another substance responsible

[1] Contribution no. 189 from the Botanical Laboratory of the University of Michigan.

for the leaves, and still another responsible for the production of flowers? Or is there but one substance, or group of substances, whose varying concentration or environmental relationships determine differentiation? Assuming for the moment the simpler and cruder hypothesis of individual formative stuffs for the various organs, we must then provide a rational explanation for their localization in and movement about the plant. They must move from their place of origin to the points where they function as organ builders.

It is obvious that grave difficulties beset one who accepts what seems, at first sight, so simple and adequate a hypothesis. The nature of the difficulties can best be understood by a review of the experimental work upon which the hypothesis of formative stuffs has been based, and by a statement of the complications of the hypothesis which become necessary if all of the facts are to be met. It is found that a more modern point of view proves in the end to be more adequate and more comprehensible.

Much of the work on organ differentiation in plants in the past has been done with *Bryophyllum calycinum* Salisb., because of its peculiar production of new shoots from the notches of the leaves. Since this plant was very well adapted to a continuance of work where former investigators had left it, the writer chose it as the material for a large series of new experiments. The use of *Bryophyllum* had the further great advantage that it would enable the work of other investigators to be reviewed, a very necessary matter, since certain results reported in the past have seemed inconsistent with one another and not in accord with the general experience of plant propagators. The historical review which forms an introduction to the writer's own work will therefore deal most fully with the past investigations on *Bryophyllum*.

Morphology and physiology of Bryophyllum

Bryophyllum is a small genus of succulent plants belonging to the Crassulaceae. The species are natives of the tropics and cannot withstand the frosty weather of the northern states. The leaves are opposite, simple or pinnately compound. The members of this genus are peculiar because of the sprouting habit of their leaves.

If a leaf is removed from the plant, and laid in a moist, warm place, young plants will grow from the notches in the margin of the leaf. This new growth starts in about five to six days after the leaf is detached. This is the easiest method of propagating these plants, for, a few weeks after the new growth is well started, the little plants can be potted off. It frequently occurs that, in greenhouses

FIG. 1.—*B. calycinum*, showing growth from notches of leaf which has been immersed in water.

where these plants are raised, leaves break off from the plant and fall to the soil beneath the plant, and growth starts from the notches of the fallen leaves. Fig. 1 shows a *Bryophyllum* plant (*B. calycinum*), while in fig. 2 are shown several leaves and the new plant growth which has come from some of the notches of these leaves.

In addition to the meristem in the notches of the leaves, each node of the stem at the axil of the leaf is potentially capable of a new plant growth. There are not really buds at these places, but unusual formations of meristematic tissue. These formations of the stem are not apparent to the naked eye, for they are buried in the recesses where the petioles emerge from the stem. There is

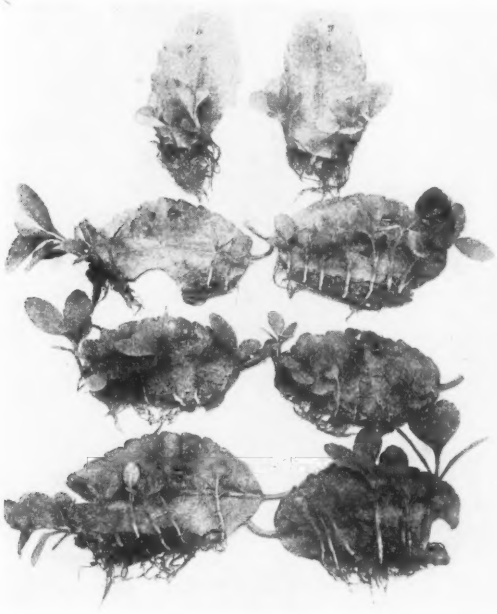

FIG. 2.—*B. calycinum* leaves, showing shoot growth from notches which were in contact with or beneath moist sand; also showing no growth from the other notches.

one on each side of the node. The location of this meristematic tissue in the leaf is easily determined, for it always occurs in the notches in the edge of the leaf, and this small sector in each notch is highly pigmented a dark brown.

These leaf groups of meristematic tissue can hardly be called buds, so that this form of propagation must be considered as growth occurring from meristematic tissue in unusual places; neither can

we use without qualification the term regeneration in describing this phenomenal growth, for it is clearly a case of vegetative reproduction and not the regeneration of a removed part. It is quite a different matter whether we remove a part of an organ and have that part replaced by a new structure of similar nature, or whether we have several new individuals arising from a part. The term regeneration has been freely and widely used in speaking of this form of propagation, and this fact will have to be kept in mind in the review of the history of the work.

Historical review

The hypothesis of formative stuffs to account for organ differentiation was originated by SACHS (9). He thought of formative stuffs as special substances potentially capable of determining the form which organs assume, and states the case as follows:

> The substances for the formation of organs (apart from the germinal stages where they proceed from the reservoirs of reserve material) are produced in the foliage leaves by assimilation, and pass out thence into other parts of the plant. So long as a vigorous main bud of the shoot is present, mixtures of substances suited chiefly for the formation of shoots pass into it from leaves, whereas the substances fitted for the formation of roots flow in the opposite direction, into the roots which already exist.

SACHS speaks of flower forming stuffs, as well as root and leaf forming stuffs, and in general of formative stuffs for each organ of the plant. These are thought of as being capable of moving or being moved about the plant.

Authors subsequent to SACHS down to about 1902 do not add notably to his conception of formative stuffs, either accepting it without amplification or neglecting it. In 1902 GOEBEL (3) published an important work on regeneration in plants in which he refers to building stuffs which he considers food material. He accounts for the shoot and root development in the leaves of *Bryophyllum* as due to correlation between the growing parts at the apex of the stem and those in the notches of the leaves. The correlation is connected with the stream flow, in some cases with the water stream, in other cases with the food stream. He gives experimental evidence to support his view. GOEBEL'S conception is essentially

the modern one, which differs from that of SACHS in that it squares with the modern physico-chemical theory.

GOEBEL also found that separation of the leaf of *Bryophyllum* from the mother plant is necessary before the growth of the leaf buds will take place. He quotes WAKKER, however, as having secured this bud growth from leaves which were not severed from the parent plant, simply by immersing the leaf in water. GOEBEL found that he himself was not able to secure the growth of the shoots from attached leaves when these leaves were kept in moist air, neither when they were overlaid with damp filter paper, nor when they were buried in damp earth. In all of his experiments it was necessary to interrupt the stream flow in some way to permit the development of the leaf meristem. By cutting the midrib of an attached leaf he interrupted the stream flow, and this leaf, although still attached, produced shoots when immersed in water.

In 1915 LOEB (5) published the first of a series of articles on the various phases of regeneration from *B. calycinum*. In this work he studied the phenomena of inhibition of regeneration in this plant. He found that the behavior was governed by the following rule:

> If an organ *a* inhibits an organ *b*, organ *b* often accelerates and favors the regeneration of *a*. This rule is best understood on the assumption that the inhibiting organ receives something from the inhibited organ necessary for regeneration.

It is interesting to note that LOEB should choose a case of vegetative reproduction in the plant kingdom on which to formulate rules governing regeneration, since vegetative reproduction and regeneration are two entirely distinct processes, probably involving different factors. He conceives of a controlling influence which a whole individual exerts upon its parts. This accounts for the fact that a part may regenerate when cut from the whole organism, but not when it is still attached to the whole. The whole inhibits regeneration from the part while the two are intact. He finds that not only the whole organism but a small part of the whole, such as a piece of the stem, inhibits growth of any portion attached to this part. A piece of the stem of *Bryophyllum* may inhibit growth from the notches of the leaf attached to this stem piece. He further found that this was true even when the leaf was cut with lateral incisions,

thus showing that the flow of material from the leaf to the stem "must have taken place along a zigzag path in the leaf." In 1916 GOEBEL (4) published a critique of LOEB's paper, showing that some of his methods were faulty, and that some of his results had been published earlier by GOEBEL. Additional experiments were given by GOEBEL to show that, when cuttings were made from the stem of *B. calycinum*, the cuttings composed of a pair of leaves with intervening piece of stem, the piece of stem did nor inhibit the formation of roots and shoots from the foliar notches.

In 1918 LOEB (6) published the results of further experiments in which he cut one leaf into as many pieces as the leaf had notches, and compared the mass growth from the notches of this leaf with the mass growth from the notches of another leaf which was not cut into pieces. Both leaves were kept on moist filter paper. The cut leaf gave new shoot growth from practically every notch, while the other leaf formed only four shoots. Concerning this, LOEB says:

> We assume that in the latter leaf the shoots which grow out first inhibit the growth in the other notches. . . . . Our contention is that this inhibition in leaf six [the uncut leaf] is due to the absorption of all of the material available for shoot formation by the four notches that happened to grow out first, thus depriving the other notches of the material needed for the growth of shoots.

In repeated experiments LOEB found that in the two cases, of slashed and unslashed detached leaves, the masses of shoot growth were practically the same weight. In the application of his rules regarding inhibition of regeneration, LOEB (5) makes the following statement:

> These rules give us some basis on which we may try to form a preliminary idea of the nature of the mechanism of inhibition. As we mentioned already, the rules are comprehensible if we assume a flow of certain (possibly specific) substances (or formed cells) from the places where the dormant buds are already to grow, or the prevention of such a flow toward these dormant buds.

The migration of formed cells from one part of a plant to another is impossible, of course, since all plant cells are surrounded by a rigid wall, making migration, such as that of leucocytes in animal tissue, quite inconceivable.

Since the rules presented by LOEB do not allow for the possibility of leaves of *Bryophyllum* growing shoots from the notches while the leaf is still a part of the whole plant, it is interesting to note the short report of Miss BRAUN (1). She cites a case in which a leaf of *B. calycinum* on a normal potted plant produced shoots without immersing the leaf in water. LOEB (7) explains the growth in this case (quite unjustifiably) on the ground that the plant in question was sick and so had not normal circulation.

In 1919 a short article reporting preliminary work by CHILD and BELLAMY (2) was published. They assume the reality of an inhibiting power of the whole on a part; that the growing tip inhibits the growth of the dormant buds in the leaf. Their method of experimentation was to interfere with or interrupt this inhibiting factor in its course between the growing stem tip and the leaf. They found that a cold compress applied to the petiole effected the block or check of the inhibiting force. In this manner virtual isolation of the leaf, so far as the inhibiting force was concerned, was effected, and resulted in the growth of shoots from the leaf thus isolated when it was immersed in water. Their conclusions were as follows:

> It appears at least highly probable that the inhibiting action of the growing tip, a leaf, or other active region of a plant, depends for its passage from point to point upon metabolically active protoplasm rather than upon purely physical transportation in the fluids flowing through preformed channels in the plant. In other words, the mechanism of this physiological correlation appears to possess at least certain of the characteristics of a transmissive or a conductive as distinguished from a purely transportative mechanism.

A close examination of the experimental work recorded on the subject reveals the fact that there is great need of a thorough study of *Bryophyllum* and the correlations involved in its process of vegetative reproduction. The laboratory findings of certain of the investigators are at variance with those of others. GOEBEL found that separation of the leaf from the mother plant was necessary before growth could be induced from the notches of the leaf, while WAKKER is credited with the converse experience. Again, LOEB'S findings confirmed GOEBEL'S, while in the still more recent work of CHILD and BELLAMY, shoot growth was secured from the

notches of the leaf while the leaf was still attached to the parent plant. Again, GOEBEL found that by making one incision through the midrib of an attached leaf, growth would occur from the notches of that leaf when it was immersed in water. On the contrary, LOEB found that a small piece of the stem inhibited growth from the leaf notches, even after he had made several incisions in the leaf. Simple as the experimentation concerned with this phase of the question may be, therein is involved the theory concerning the movement of formative stuffs through the conducting systems of the plant. These are but examples of several instances of variance in the experimental data at hand on the subject, hence the need of a comprehensive study of the subject. It is hoped that the following experiments, frequently repeated and substantiated, may form a basis for conclusive and constructive ideas on the subject.

An entire, normal plant of *B. calycinum* was used, the crenate leaves of which, both simple and compound, are known to be potentially capable of the growth of new plants from the marginal notches. It is generally known that the leaves are disconnected from the parent plant before this new growth occurs, so the question arises, is there a correlation between the severance of the leaf from the plant and the new growth? If so, what is this correlation?

Further experiments on Bryophyllum calycinum

EXPERIMENT I.—The first question which naturally arises is: Does the leaf in general possess the power of new plant growth, or is this power definitely located in certain parts of the leaf? It is not an assumption that leaves of *B. calycinum*, when removed from the plant and placed on a moist substratum in a warm place, will grow new shoots from several, if not all, of the notches of the leaf.

Twenty leaves were removed from plants and the petioles cut from the leaves. From each notch of each leaf a small sector of the leaf tissue was removed by means of a sharp razor. Caution was exercised in the removal of this tissue to insure the removal of the very smallest portion of the leaf necessary, and still withdraw all of the meristematic tissue at these points. The comparative size

of the sections of tissue removed may be seen in figs. 3 and 4, the latter showing uncut notches. These twenty leaves were divided into two sets of ten each. Those of one set were implanted on moist sand, those of the other set were suspended vertically in damp chambers with the petiolar third of each blade in water. Except for the growth of root from the base of the midrib, as shown

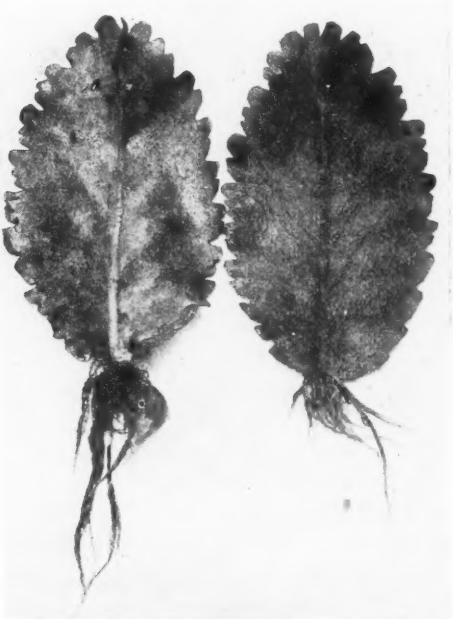

FIG. 3.—*B. calycinum* leaves from which meristematic units have been removed, showing growth of roots from base of midrib; also showing lack of foliar shoots.

in fig. 3, there was no growth from these leaves. The experiment was continued for several months, in which time several of the leaves rotted. The small sectors which had been removed from the periphery of the leaves were placed in a damp chamber, and the great majority started plantlets in a few days.

This experiment not only shows the definite location of the meristematic tissue in the leaf, but also indicates that the potentiality for growth of shoots from the leaf is localized in these small

sectors of meristematic leaf tissue which were removed. Further, that the factor responsible for growth is something formed and centralized when the leaf is on the plant, and that a subsequent or continuous formation of this factor does not occur, for, if it did so, growth would have occurred subsequent to the removal of the sectors of tissue.

FIG. 4.—Leaf of *B. calycinum*, showing growth of roots from base of petiole; leaf continuing to live normally, and no evidence of germination of meristematic units in notches.

EXPERIMENT II.—Since there are meristematic units in the notches of the leaves of *B. calycinum*, the questions arise, what factors control the growth, or the dormancy, of these units? Why is it that the units do not grow while the leaf of which they are a part is attached to the normal plant? Does the plant or any organ of the plant exert an inhibiting influence on these units of meristematic tissue? Are they dependent upon certain specific substances or formative stuffs for their growth, which the plant, or any organ of the plant, consumes at their expense? It has previously been seen that the opinion of some is that the growth of these units is dependent upon certain specific substances or formative stuffs, and that in the normal plant this formative stuff is used by the other growing parts of the plant with the result that the meristematic units of the leaves remain dormant. It has further been stated by some that not only the other growing parts of the plant inhibit this growth from the leaf notches, but even parts of the plant, such as a piece of a stem attached to a leaf or the axillary buds of the piece of the stem, may inhibit. As the reason for this was not clear, such experiments as would throw some light on the subject were repeated many times.

Twenty leaves, each with a piece of the stem attached (fig. 5), and with axillary buds removed from each stem piece, were suspended in a damp chamber for the purpose of noting what, if any, inhibitory effect this stem piece would have over the growth of the meristematic units of the leaves. For each leaf of this culture, its normally opposite leaf without a stem piece attached was also suspended in the damp chamber for control observation. It was surprising to note with what uniformity growth occurred from all the leaves in the damp chamber. There was no noticeable difference

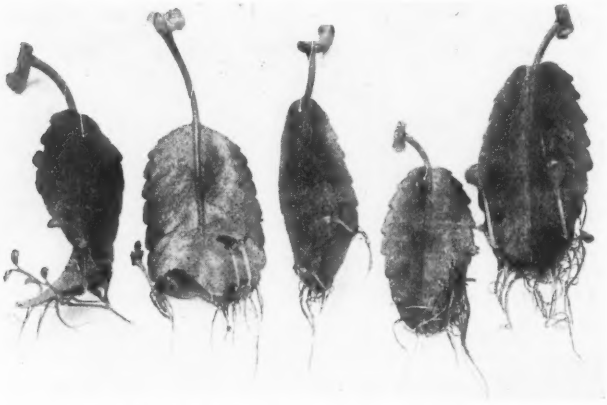

FIG. 5.—Leaves of *B. calycinum*, with stem pieces attached to petioles, with both axil meristems removed, showing growth of foliar shoots.

between the growth from the leaves with the stem piece attached and from the control leaves. Figs. 5 and 6 show ten of these leaves with the stem piece attached in each case and with each leaf showing shoot growth from the notches nearest the water. A similar experiment was tried with leaves having the stem pieces attached and having neither stem bud removed. Growth occurred from the opposite axillary bud, but evidently with no inhibiting effect upon the meristematic units of the leaves, for each leaf gave growth from the notches nearest the water as in the previous case, and as did the control leaves in this experiment. Fig. 7 shows five leaves of this experiment with the shoot growth occurring

simultaneously from the notches of the leaves and the opposite axillary buds. A third culture was set up, repeating the two

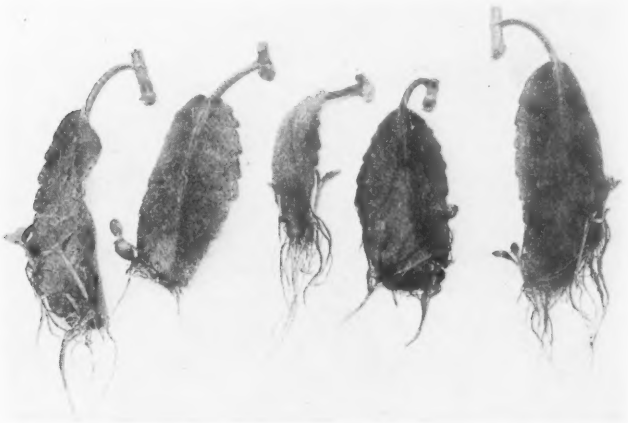

FIG. 6.—Leaves of *B. calycinum*, with stem pieces attached, with only opposite axil meristem removed, showing growth of foliar shoots.

FIG. 7.—Leaves of *B. calycinum*, with stem pieces attached, with neither axil stem bud removed, showing growth from leaf notches as well as from opposite axil meristem.

foregoing experiments at the same time in the same damp chamber, and having a control leaf for each case. The growth in this culture confirmed the results of the two previous experiments.

All of these experiments, however, with their leaves suspended petiole upward in moist air above water, entail unnatural and unusual conditions. The leaf severed from the plant and placed horizontally on a moist substratum will, in the same length of time (about five to six days), show growth occurring from the notches. Also the axillary stem bud will grow under these more usual conditions. If the growth from the stem bud inhibits growth from the leaf it will surely do so under these conditions. Leaves with stem pieces and axillary buds intact were laid on moist sand, the leaf as well as the stem piece being in contact with the moist sand. In

FIG. 8.—Leaf of *B. calycinum*, showing that root growth occurs from meristematic units in notches of leaf, regardless of shoot growth from opposite axillary meristem.

every case the axillary stem bud grew, and in every case there was shoot growth from some of the notches of the leaf. Of course control leaves were used, being laid horizontally on the sand as the others were. In some cases there were a few more shoots on the control leaves than were on their respective leaves with stem pieces attached, but the converse was also true. There seemed to be no regularity about the shoot growth. Fig. 8 shows a leaf of this experiment.

The writer is thus unable to verify the work of LOEB, which indicates an inhibiting influence that the stem and the growth from the opposite axillary bud may have on the leaf shoots. LOEB has stated that it is the opposite axillary bud which inhibits the growth from the leaf. The writer knows of no morphological

reason why the opposite bud growth should inhibit and not the one at the base of the petiole of the leaf in question. Fig. 9 shows the growth from the axillary stem bud at the base of the petiole. It is very evident that this plant growing at the base of this petiole is fed from the leaf; there is no other source for food. This small plant, therefore, drawing its food from the leaf, should draw as strongly on the hypothetical formative stuffs of the leaf as any growth which may occur from a bud on the opposite side of an intervening stem, yet we see no signs of inhibition of growth from the leaf notches in fig. 9.

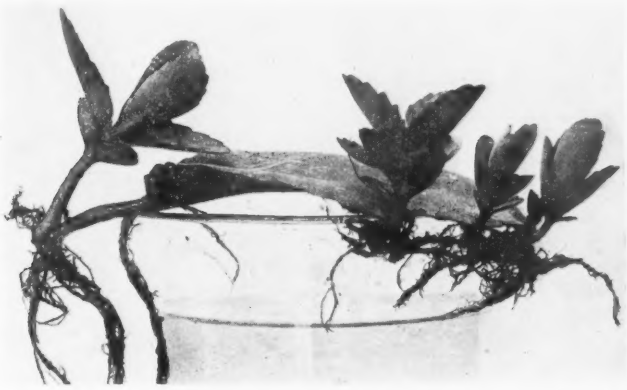

FIG. 9.—Leaf of *B. calycinum*, showing growth from meristem at base of petiole and from meristematic units of leaf.

EXPERIMENT III.—It has been asserted that growth at the apex of the stem of *Bryophyllum* inhibits growth from the leaf notches. LOEB explains the mechanism of this inhibition as a "sucking" of formative material away from the notches of the leaves by the growing parts at the apex, where the material is used for the development of new foliage. For the purpose of testing this hypothesis, the writer prepared five cultures of apical stem pieces bearing from one to three pairs of leaves. One specimen had one pair of mature leaves besides the small immature apical leaves, two others had two pairs, while the others had three pairs of the maturer leaves besides those which were immature. The five specimens were planted in

moist sand, with the base of the stem in each case inserted in the sand. As shown in fig. 10, each stem developed a vigorous growth of roots at the base. After the roots were well developed, sand was built up to the large leaves of each specimen, thus bringing the meristematic units of each leaf in contact with the moist substratum. If it were true that the growth of the new apical leaves and the apical bud caused inhibition of growth from the notches of the older leaves, we might surely expect such inhibition in these five

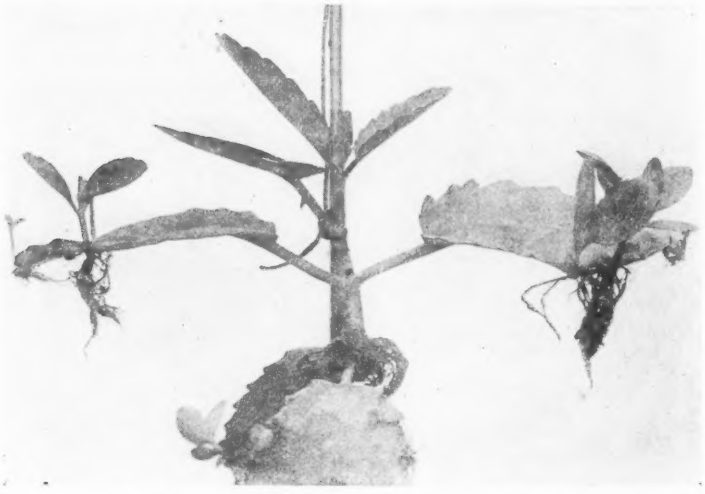

FIG. 10.—Growing tip of main stem of a *B. calycinum* plant, showing root growth from base of stem and shoot growth from notches of large leaves.

specimens, for in each case we have reduced the number of leaves this growing section might draw upon by cutting away, as we might say, the rest of the plant. Such was not the result, however, as may be seen in fig. 10. All specimens behaved in a manner similar to that shown in the figure. The specimen with one pair of large leaves gave shoot growth from 28 notches. One of the specimens with two pairs of large leaves gave shoot growth from 31 notches, while the other gave growth from 32 notches. One of the specimens having three pairs of large leaves gave shoot growth from 32 notches, while the other gave growth from 45

notches. It becomes evident, therefore, that at least in this case the growing apical parts of the plant did not inhibit growth from the notches of the leaves. In general we know that throughout the plant world we have dormant buds in plants, and that the awakening of these dormant buds often follows the removal of some growing part, but to say that growth in certain parts is the cause of dormancy in others may perhaps not be the correct analysis of the correlation.

EXPERIMENT IV.—Having segmented the plant into parts and having studied these parts, we are led to the conclusion that, at least in these parts of the plant, there is no one organ or group of organs inhibiting the growth of the meristematic units of the leaves under conditions under which moisture is supplied to the leaves externally. The question then is, how does the plant as a whole inhibit growth from the leaves? To answer this a set of cultures was prepared, using entire plants with one leaf of each immersed in a basin of water. Of course the immersed leaf in each case was not removed from the plant nor injured in any way. Fig. 1 shows a plant after the leaf had been immersed in water some days. In every case the leaf immersed in water gave shoot growth from some of its notches. This figure is a typical example of the experiment. GOEBEL (3) has reported that he was unsuccessful in obtaining shoot growth from the leaves in this manner, but he quotes WAKKER as securing such results. CHILD and BELLAMY (2) reported growth secured from the leaf while it was immersed in water and still attached to the plant. They explained this growth as due to the interference with the conducting or transmitting system by the cold application they used.

EXPERIMENT V.—A set of plants similar to those in experiment IV was placed in a dark room, which was ventilated with air forced in by a blower from the adjacent greenhouse. The plants were watered daily, but no water was placed in contact with any of the leaves. In other words, the plants were continued in their natural conditions except that they were deprived of light. In two weeks, growth of shoots was apparent from notches of many of the leaves. Combined with the data of experiment IV, these results point to the probable conclusion that the plant as a whole does not cause the dormancy in the notches of the leaves.

EXPERIMENT VI.—If the dormancy of the growing points in the notches of the leaves were dependent upon and a result of inhibiting factors from the growing parts of the plant, and we sever some of the leaves from the plant, we then remove this inhibiting force from these leaves and should obtain growth from some of the notches of the leaves, providing they are placed in a moist and light place.

After isolating several leaves from their stems, root growth was started from the base of the petiole of each by implanting the petioles in moist sand. The leaves were in an upright position and continued thus in a normal, healthy condition for six months without the occurrence of growth from any of the leaf notches. In this case it must be borne in mind that the lamina is not in direct contact with the moist sand, but the fact that the leaves continued apparently to perform their normal metabolic processes for six months (or indefinitely, for the experiment was not permitted to go longer), and at the same time showed no tendency toward producing new growth from the notches, is a result worthy of attention. In this case we have leaves similar in all respects to the leaves on the whole plant, except for the fact that they are no longer a part of the plant; they are no longer subjected to any inhibiting force exerted by the plant, and still the meristematic units remain dormant. We have severed the leaf, or the part, from the whole and still have dormancy. May we not therefore discard the whole (the plant in this case) as the source of the factor responsible for the dormancy of the meristematic units of the leaves? We have removed the plant from the leaf and still have dormancy; therefore, we have not removed the factor causing dormancy.

Continuing the experiment, at the end of six months the midrib of each leaf was cut through, the leaf being otherwise undisturbed. Three weeks later shoots had grown from some of the notches of each leaf; for example, 21 out of a possible 29 notches of one leaf produced shoot growth after the midrib was cut.

EXPERIMENT VII.—If we are to assume that shoot growth from *B. calycinum* leaves is dependent upon formative stuffs within the leaves, and that this formative stuff is transportable about the leaf, we may agree with LOEB that the growing of a certain number of

these units of meristematic tissue will withdraw from the leaf all of the formative stuff, and so deprive the remaining units of formative stuff necessary for their development. On the contrary, if good growth is secured from some of the notches of a leaf and subsequently good growth takes place from the remaining, or a portion of the remaining, notches of the same leaf, we cannot agree that his hypothesis applies, unless we assume that new formative stuff is continually being produced in the isolated leaf, and even in that case his "suction" hypothesis would hardly apply. By placing a portion of each leaf in contact with moist sand, good growth (plantlets 10 cm. high) was secured from those notches in contact with the moist sand, each leaf being severed from the parent plant. At the same time there was no growth from those notches which were not in contact with the moist sand. These new plantlets were then severed from the leaves, and the leaves inverted and again implanted in the sand, this time causing the heretofore dormant units to be in contact with the moist substratum. Within the usual time, eight days, there was a second crop of plantlets from these leaves, the growth again occurring from those notches in contact with the moist sand.

Another set of leaves was treated in a somewhat similar manner, but in this case, after those buds which were in contact with the sand had developed plantlets reaching 10 cm. or more, the leaves were not removed from the sand, but that part which extended free in air and the buds of which had remained dormant was brought in contact with the sand by slightly bending the leaves and building up the sand. The first plantlets to grow were not removed from these leaves. Again, within eight days, the heretofore dormant units of meristematic tissue unfolded into plantlets, as did the first to be favored with contact with the moist substratum. The first plantlets to grow did not deplete the leaf of that which was necessary for the growth of the other buds, nor did the growth of the units depend upon anything which had to be furnished from the leaf and which was subject to consumption or depletion by the first units to grow.

EXPERIMENT VIII.—Another series of isolated leaves was treated in the following manner. Some of the leaves were implanted with

only the very basal portion of the leaf blade beneath the moist sand. Others were almost entirely buried, and a grading series provided all possible intermediate conditions. Within ten days all of the notches of all of the leaves which were in contact with or beneath the sand gave shoot growth, while none of the notches above the sand did so. Fig. 2 shows a set of leaves treated in a similar manner, only some of the leaves were placed with their sides in the sand. Only those parts of the leaves which had been in contact with or beneath the sand showed growth. Here we have two unusual conditions in operation, one being the presence of water, the other being the absence of light.

EXPERIMENT IX.—To study the effects of the absence of light on the shoot growth from the notches of leaves of *B. calycinum*, a series of isolated leaves was treated in the following manner. One set of leaves was placed in a dry position before a south window; another set was placed in a dry dark box which was entirely light proof. Within ten days every notch of all the leaves in the light-proof box gave shoot growth, while only a very few of the notches of a few of the leaves in the light did so. As might be expected, the shoots which grew out in the dry box did not survive long. One may not assume, however, that the moisture of the air was the same in both sets of plants.

EXPERIMENT X.—It has been said by LOEB that approximately equal masses of new shoots are produced by equal masses of sister leaves, and from his work one may infer that "the material available for shoot formation" is of a specific nature. If such is the case, the mass of shoots affords a quantitative measure of the relative amounts of formative stuffs. If the mass of the leaf available for growth of shoots from the notches does not affect the number of notches giving initial growth, but on the contrary controls only the subsequent quantitative development of the plantlets, then the hypothetical "formative stuff" which is furnished the plant from the leaf simply fulfils the function of food stuff. If the number of notches to produce plantlets were dependent upon the mass of leaf tissue capable of being drawn upon by the meristematic units in the notches, one might conclude that the substance used in the initial formation of the shoot was derived from the leaf in general, and was specific, movable, formative stuff.

With this question in mind, a series of five sets of leaves was prepared, three similar leaves in each set, the total number of notches in each set being the same. One set was left entire. In the remaining four sets, a portion was cut from the central part of each leaf, varying from one-fifth of the leaf mass in one set to four-fifths in the last. All five sets were placed in glass damp chambers exposed to daylight. If growth from the notches of these leaves were dependent upon a limited amount of highly mobile formative material drawn by the meristematic units, or furnished to them, from the general mass of the leaf, the less the mass of the leaf the greater would be the inhibiting effect of the first buds to grow on the remaining ones of that leaf; the greater the mass, the greater the possibility of many buds starting. If, however, only the subsequent development of the plantlets, not their initial number, were decreased by the decrease of mass of leaf tissue, the question would appear to be one of food supply only.

Listing the sets numerically, beginning with that in which the leaves were left entire and ending with that set from which the greater portion of the middle of the leaf was removed, the number of notches giving initial growth was as follows:

Set 1 gave initial growth from 38 notches, out of a possible 60
Set 2 gave initial growth from 32 notches, out of a possible 60
Set 3 gave initial growth from 44 notches, out of a possible 60
Set 4 gave initial growth from 32 notches, out of a possible 60
Set 5 gave initial growth from 35 notches, out of a possible 60

There appears to be no correlation whatsoever between the mass of the leaf and the number of meristematic units giving initial growth of plantlets, but in the further development of the plantlets it was very apparent that there was correlation between the mass of the leaf and the size of the plantlets. Those of the first set were larger than those of the second, those of the second larger than those of the third, etc.

Discussion

SACHS predicates different kinds of formative stuffs, a kind for each organ of the plant. These are capable of moving or being moved about the plant. GOEBEL speaks of building stuffs which he considers to be food material. He accounts for the shoot and root development in the leaves of *Bryophyllum* as due to correlation

between the growing parts at the apex of the stem and those in the notches of the leaves. The correlation is connected with the stream flow, the water stream in some cases, the food stream in other cases. In the application of his rules regarding inhibition of regeneration, LOEB assumes the inhibition to be due to "a flow of certain (possible specific) substances (or formed cells) from the places where the dormant buds are ready to grow, or the prevention of such a flow toward these dormant buds." The migration of formed cells from one part of a plant to another is impossible, of course, since all plant cells are surrounded by a rigid wall, making such migration as that of leucocytes in animal tissue quite inconceivable. These writers assume the existence of an inhibiting factor in the plant, functioning on the meristematic units of the leaves.

If we are to assume that shoot growth from *Bryophyllum* leaves is dependent upon formative stuff within the leaves, and that it is transportable about the leaf, we may agree with LOEB that the growing of a certain number of shoots from the leaf will withdraw from the leaf all of the formative stuff present, and so deprive the remaining growing points of formative stuff necessary for their development. This hypothesis allows for only one crop of shoots from a *Bryophyllum* leaf, for the first crop consumes all the substances required for the growth of shoots, automatically preventing any further shoot growth from the leaf. If a second or third crop of shoots is possible from the leaf, therefore, we cannot agree that his hypothesis applies.

As has been shown by experiment VII, a number of *Bryophyllum* leaves were so placed on moist sand as to permit growth from only half of the notches of each leaf. After the plantlets grown from these notches were well developed, one might have expected a depletion of formative stuffs within these leaves caused by the growing of these plantlets, but this was not found to be the case. A second crop of shoots was grown from the opposite side of these same leaves after removing the first. Again, as shown in the same experiment, a second crop can be grown equally well from a set of leaves without removing the first crop. Corresponding results may be obtained at any stage of growth of the foliar plantlets, from the initial stages of unfolding to the larger sizes several weeks old.

It appears evident, therefore, that growth from a portion of the meristematic units was not the cause of continued dormancy in the other growing points of the same leaf. It is further evident that the plantlets first to grow did not deplete the leaf of that which was necessary for the development of plantlets from the other notches.

It is frequently found that the plantlets which grow under like conditions from the same leaf show great variation in development, and some may even die, apparently due to other plantlets from the same leaf inhibiting their growth. Rather than attribute this inhibiting effect to the consumption of formative stuffs by some plantlets at the expense of others, however, is it not more probable that the nutrient supply only is used by those of more early growth at the expense of the later ones? That initial growth can be obtained from all the notches of the leaf is shown by experiment IX, but for a leaf to be able to support the subsequent development of plantlets from all its notches would appear to depend upon the availability of food.

LOEB has shown that approximately equal masses of new shoots are produced by equal masses of sister leaves. This is not a new discovery in plant physiology. It is well known that equal masses of plant tissue can be grown from equal amounts of endosperm and other food material. From LOEB'S work, however, one may infer that the material available for shoot formation is of a specific nature. If such is the case, the mass of shoots affords a quantitative measure of the relative amounts of formative stuffs, but when, as shown in experiment X, we find that there is no correlation between the amount of leaf mass and the initial shoot growth, we are forced to the conclusion that the initial growth of the meristematic units of the leaves of *Bryophyllum* is not dependent upon specific substances derived from the leaf in general.

Passing from the isolated leaf as a unit, we may now consider briefly the leaf and a piece of the stem as a unit separated from the plant. With many repetitions of the various experiments with this unit, with and without the axillary buds, the writer was unable to verify LOEB'S observations on similar experiments. As is shown by experiment II and figs. 5–7, 9, 10, there was growth from leaf

notches when water was present and available, regardless of growth from axillary buds or the presence of a piece of a stem.

Normal growth at the apex of the stem is quite a different thing from the growth of adventitious buds in parts of the plant, but, as shown in experiment III, even this growth at the apex does not inhibit the growth of plantlets from notches of the leaves when moisture is directly available to the meristematic units of the leaves. It may be possible that if the leaves have a limited amount of water to draw on, the active growing parts at the apex of the plant will draw so heavily upon this limited amount of water as to deprive the meristematic units of the leaves of their required amount for proliferation.

CHILD and BELLAMY start their work with the assumption that the growing tip does inhibit growth from other parts of the plant, and with this assumption they attempt to block this inhibiting factor in its course to a leaf of *Bryophyllum* by the use of a low temperature application to the petiole of the leaf in question, then by immersing the leaf in water the notches of the leaf give growth. In view of the fact that shoots will grow from leaves of *Bryophyllum* when immersed in water without the cold application, as is shown in fig. 1, can we be certain that their method of applying the cold application is responsible for the shoot growth they observed? Although some writers report that they are unable to secure shoot growth from leaves when immersed in water if intact with the plant, it is difficult to understand why this should be so; for it frequently occurs in greenhouses that such leaves, while in normal position on the plant, develop shoots without being immersed in water. Assuming again that the dormancy of the growing points in the notches of the leaves of *Bryophyllum* is dependent upon and a result of inhibiting factors from the growing apex of the plant, or from any part of the plant, or from the plant as a whole, it is obvious that these inhibiting factors are removed from influence in the leaf by severing the leaf from the plant. It is then to be expected that plantlets will grow from the notches of these leaves, since the factors which had caused their dormancy have been removed. In Experiment VI, however, it is shown that the meristematic units in the notches of the leaves of *B. calycinum* may be

kept in a dormant condition indefinitely without the influences of the inhibiting factors of the parent plant. It is found that leaves severed from the parent plant may continue in an apparently normal condition indefinitely, providing water supply is made possible by the growth of roots from the petiole of the leaf.

Let us return to the original assumption of some writers that the reason the meristematic units in the notches of the leaves remain dormant while the leaf is intact on the plant is that the plant inhibits the growth from the notches, because the growing part of the plant uses the formative material required for the growth from the notches of the leaf. We here remove the leaf from the plant, then remove the plant from the leaf, and still have continued dormancy. The cause of dormancy has been removed and still dormancy continues. In the face of this evident inconsistency, we are forced to the conclusion that we must allow for at least another cause of dormancy. Moreover, we have moved the possible seat of the factors for dormancy from the plant to the leaf.

The culture in question, that of an isolated leaf continuing an independent existence without the unfolding of the foliar shoots, is very interesting and of importance. Its great value lies in the fact that it eliminates many of the greatest difficulties of the problem. In place of LOEB'S question, "What are the forces in the whole which exercise control over the part resulting in the prevention of regeneration?" we now ask, "What are the forces in the part which result in the dormancy of the meristematic units of this part?" The problem now involves the individual leaf, dormancy, and water. A new factor, namely, water supply, engages our attention instead of the hypothetical influence of the entire plant. Experiment VI shows that after six months of continued independent and apparently normal existence, the period of dormancy was broken by the cutting of the midrib, and hence, by removing the factor of water supply. Twenty-one out of a possible twenty-nine meristematic units grew after the midrib was cut, after the water was withheld from the conducting channels of the leaf. By cutting the midrib the water relations of the leaf were modified, and this probably set up a series of physical and chemical changes. To these changes, whatever they may be, we would ascribe the phenomena observed.

In his book on regeneration, MORGAN (**8**) makes the following statement:

> There is another point which has been, I think; too much neglected, viz., that the production of food stuffs is itself an expression of changes taking place in the living tissues, and if the structure is changed so that it no longer produces the same substances it may then lead to the development of different kinds of organs. The difference in the regeneration of the apical and basal leaf of *Begonia* may be due to some difference in the structure of the protoplasm. The greater or smaller amount of starch produced in these leaves may be only a measure of, and not a factor in the result.

Attention is here focused on the protoplasm. Consider again the case previously mentioned, in which growth was obtained from a portion only of the notches of an isolated leaf, and later the remaining notches of the same leaf were induced to sprout by bringing them into contact with the moist sand. Again there is modification in the water relations of the leaf, and even parts of the leaf, and the probable physical and chemical changes in the protoplasm.

In experiment VIII a number of leaves provided a grading series so implanted in moist sand as to provide leaves with but a few notches beneath the moist sand, leaves nearly entirely buried, and intermediate conditions. In all of the leaves only those notches which were in contact with or beneath the sand gave rise to shoot growth. Fig. 2 shows a set of leaves of this culture. The buried portion only shows growth. There are two factors of possible influence in this case, (1) the presence of water, and (2) the absence of light. Both are capable of bringing about physical or chemical changes within the leaf.

1. *Presence of water.*—Several experiments have been cited wherein the presence of water appeared responsible for the growth of the dormant buds in the leaf of *B. calycinum*. Fig. 1 shows a culture after one leaf in a normal position on the plant had been immersed in water. The culture was grown in the greenhouse. Numerous cases have been cited by other investigators in which water was a factor in the development of growth from the leaf notches. It is evident that the presence or availability of water is of prime importance in this problem. It has been shown that water is perhaps the deciding factor in the case. One other is to be considered, however, the absence of light, and it may even be shown

that both water and light are only of secondary importance, influencing indirectly the resultant growth of the meristematic units of the leaves.

2. *Absence of light.*—To test the effect of light, or the absence of light, two sets of leaves were prepared. One set was placed in a dry location before a south window, the other in a dry lightproof box. As shown by experiment IX, the absence of light from the leaves, although in a dry place, resulted in so extensive a germination of the meristematic units that shoots sprouted from every notch of every leaf in the culture. In the same length of time there were but very few shoots started from those leaves before the window in the light, these also being without water. In experiment V is mentioned the fact that leaves still attached to the plants in the dark room produced shoots from some notches. The production of shoots in this case was by no means as extensive as that from the leaves in the lightproof box. This difference might be expected when we realize that physical or chemical changes would be less complete and slower in the leaves enjoying the transpiration and connection with the plant than in isolated leaves deprived of water. It appears, therefore, that, although the presence of water seems to be of great importance in this problem, the absence of light may be of equal importance. The single detached leaf with roots in moist sand is furnished water and nutrient, as is the leaf of the normal plant. In such a leaf, one should not expect physical or chemical changes other than those occurring in a leaf on a normal plant, but by cutting the midrib of the leaf having its own connection with the moist substratum we cut off the water supply and should expect these changes to occur in the leaf. Throughout all of the experiments reported, wherein foliar shoot growth has been recorded, this growth has followed some condition in which the leaf was subjected to conditions which would result in the metabolic changes mentioned.

Owing to the fact that most of the experimental work on this problem has been done with the use of water, the rôle of water becomes, perhaps, unduly magnified. We must not overlook the fact that the most abundant case of initial shoot growth from leaves occurred in the dark, in the lightproof box. In this culture are

provided the conditions which would bring about the most rapid and complete changes within the leaf, darkness and dearth of water. Use but one of these conditions alone and the changes will be slower and less complete. For example, the leaves lacking water, but in the light, would naturally continue normal metabolism longer than if the light had been shut off also. The great importance of the absence of light is lost sight of, because cultures of plant tissue do not lend themselves readily to dark room conditions. The starved plant in the dark is under very different metabolic conditions from the plant in the light, and this very fact emphasizes the importance of the effect of darkness.

There is one phase of this problem which should not be overlooked. It has been reported and frequently noted that leaves of apparently normal *Bryophyllum* plants will grow shoots from their notches while the leaf is still attached to the plant. Since such a case, reported by Miss BRAUN (1), conflicted with LOEB'S "suction" hypothesis, he explained the growth on this particular plant as being due to the fact that the plant was sick and so had not normal circulation. It is a question whether such a plant, although it may be sick, is less healthy than portions of plants which have been cut off for experimental purposes. But grant that the plant is sick. What is the significance of that? Merely that the metabolic condition of the plant had changed, and if our inferences have been correct, plants such as this, sick, or undergoing metabolic changes, may be expected to show growth of shoots from the notches of the leaves. In cases of this kind the appearances of shoots on the leaves is not necessarily an indication that the conducting tracts of the plant are interrupted, but it is, perhaps, only an expression of the metabolic condition of the organ.

In formulating a conclusion, let us visualize the growing meristem in the notch of the leaf of *B. calycinum*. It is a small unit of meristematic tissue, formed in the notch of the growing leaf, surrounded by the normal metabolic processes of the growing leaf, and as long as the leaf continues to function normally and there is no change in the metabolic processes of the leaf, the meristematic unit undergoes no change. Interrupt the metabolic conditions of the plant, or the leaf, as has been done in many ways in the pre-

ceding experiments, and the meristematic units unfold and give rise to plantlets. External changes may bring about physical or chemical changes within the leaf. Water, absence of light, or even cold compress on the petiole may initiate a concomitant series of physical or chemical changes within the leaf. It is to these changes, whatever they may be, that we would ascribe the proliferation of the meristematic units.

In this analysis of *B. calycinum* it is found that the growing points in the notches of the leaves are small independent units, independent of other organs of the plant, and dependent only upon their imbedding media, as we might say. The indications are that there is no formative stuff or specific substance withheld from these growing points or fed to them.

Summary

1. The growth of foliar shoots in *B. calycinum* is possible only from the preformed buds in the notches of the leaf.

2. When these *Bryophyllum* plants are growing under normal conditions the buds in the leaves lie dormant.

3. There are no indications that this dormancy is due to the fact that these units are deprived of formative stuffs or specific substances, the availability of which would cause their germination.

4. The plant or any organ of the plant does not exert an inhibiting influence over these meristematic units.

5. The dormancy or the germination of these units is an expression of the metabolic condition of the organ of which they are a part.

6. The germination of these units is probably due to physical and chemical changes within the organ of which they are a part. Any factor or group of factors working together which set up this series of changes are indirectly responsible for the growth of the foliar plantlets or those from the axillary buds.

7. One of the conditions which starts the growth from the meristematic units of the leaf is very moist air or water in contact with the leaf.

8. The absence of light also brings about a condition which starts this growth.

The research whose results are here recorded was done at the University of Michigan under the advice of Professor F. C. NEWCOMBE, for whose guidance the writer wishes herewith to make due acknowledgment.

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FOOD OF THE OYSTER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 303

G. W. MARTIN

(WITH NINE FIGURES)

Introduction

The common oyster of the Atlantic coast of North America, *Ostrya virginica* Gmelin, occurs from the St. Lawrence River to Central America. While oysters can endure rather a wide range of conditions as to salinity and temperature, they thrive best "in shallow bays and estuaries of rivers, where the water is warmer and less saline than that of the sea, and where there is also a greater abundance of suitable food" (**27**). The studies here reported were conducted in New Jersey, where both Barnegat Bay in the east and Delaware Bay on the west include large areas suitable for oyster culture. The field work was done at the oyster culture laboratory of the New Jersey Agricultural Experiment Station at Barnegat, New Jersey. Much of the examination of material and analysis of the data were done at the Hull Botanical Laboratory of the University of Chicago, under the direction of Professor HENRY C. COWLES. For much information and many valuable suggestions based on his wide experience in oyster work, as well as for material collected during the autumn, winter, and spring of 1921–1922 at different points in New Jersey and forwarded to Chicago, acknowledgment is due DR. THURLOW C. NELSON, biologist in charge of oyster investigations in New Jersey.

In order to explain the conditions under which the experimental work was performed, a brief description of the surroundings of the laboratory at Barnegat is appropriate. The laboratory is situated on a point on the north shore of Barnegat Creek, just where the latter widens out into a broad, shallow salt lagoon (fig. 1). Barnegat Bay is somewhat more than half a mile to the east, the lagoon being separated from the Bay by the narrow strip of sandy beach and salt marsh which forms the northern extension of Conklin's

Island. In the lagoon are several small marshy islands. The muddy bottom, nowhere more than five feet under water at ordinary tides (except in the dredged channel), is nearly all adapted for oyster culture, although because of its shallowness it is likely to be

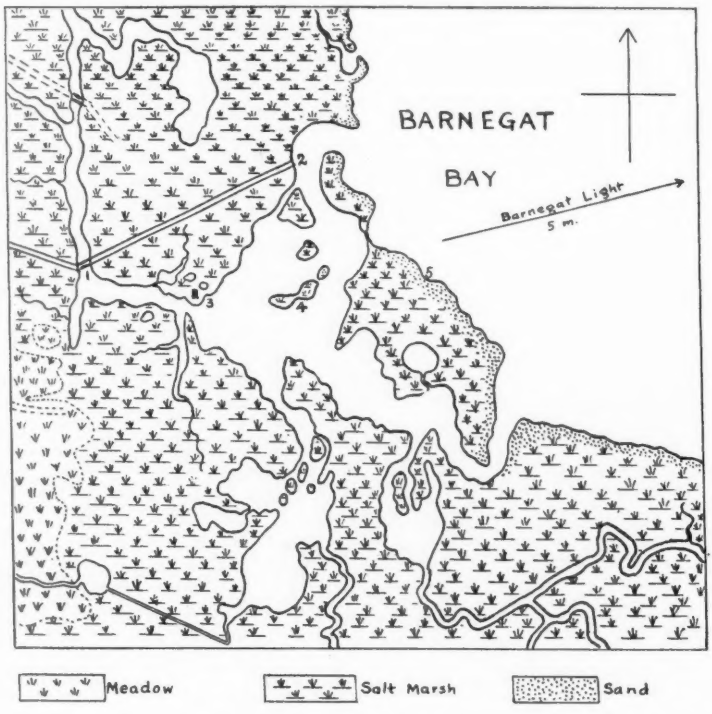


FIG. 1.—Vicinity of Oyster Culture Laboratory, Barnegat, one and one-half miles from east to west: 1, inner dock; 2, outer dock; 3, laboratory; 4, Cat Island; 5, Conklin's Island.

gutted by ice during severe winters. It is somewhat sparingly covered with oysters, as these grounds are used mainly for securing oyster seed.

The adjacent low marshy lands are typical of the salt marsh areas of the northeastern United States. The wetter parts of the meadows are dominated by *Spartina glabra* var. *pilosa*. In the

drier portions, usually cut for salt marsh hay, this grass is replaced by *Spartina patens*, *Juncus Gerardi*, and associated species. Mats of algae, mainly *Vaucheria*, *Cladophora*, and various blue-greens, often cover the ground. This growth, however, seems to contribute little except pollen to the oyster's food supply. A conspicuous feature of the bay shore is the dense windrow of dead *Zostera* cast up by the waves. This mass of decaying vegetation is often three feet or more in thickness and ten or fifteen feet in width.

Scattered among the marshes are numerous shallow brackish ponds, the larger of them connected by natural creeks with the lagoon or bay; the smaller usually without a natural outlet, but commonly connected by drainage ditches dug in the course of mosquito extermination work. Many of these ditches are now almost choked up, and some of the smaller ponds seem never to have had them. As a result, it is only after heavy rains or during periods of exceptionally high tides that such ponds have any connection with the open water. Their salinity is usually somewhat higher than that of the lagoon, being highest, of course, after periods of dry weather. Their temperature fluctuates much more than that of the larger bodies of water. The important feature of these ponds is the great abundance of plankton in them, which, as noted later, makes them important sources of food organisms utilized by the oyster.

The water of the creek at the laboratory ordinarily varies in density between 1.010 and 1.016, but may sometimes fluctuate rapidly beyond these limits. Especially after violent thunder storms, the upper layer of water may for a time become practically fresh. The water in the lagoon near Cat Island seems to vary between 1.012 and 1.018 as a rule, and was not observed to go below 1.008. Southerly winds, driving the water into the northern end of the bay, lower the density by increasing the importance of the fresh water increment. Conversely, northerly and easterly winds increase the density. The bottom of the bay is very largely covered with *Zostera*, accompanied by masses of algae, such as *Spyridia filamentosa*, which occurs in dense masses, and a number of red algae of subordinate importance, such as *Ceramium strictum*, *Polysiphonia Harveyi*, and other species belonging to these and

other genera. In the shallower parts of the bay, beds of *Ruppia maritima* occur. Very little *Zostera* grows in the lagoon, but much is washed in with the currents. A little *Ruppia* occurs, but for the most part the bottom is either without larger plants, or is covered, in the warmer months, with a mass of *Ulva*, *Cystoseira*, and *Spyridia*, accompanied by *Agardhiella tenera*, *Ceramium strictum*, and *Polysiphonia* spp., some of these growing naturally in the lagoon, but the greater part being washed in from the bay. Masses of *Spirulina tenuissima*, growing around a matrix of decaying seaweed, are common, and *Enteromorpha intestinalis* grows abundantly on shells and on wooden stakes and piles.

From this description, it will be seen that conditions in the lagoon at Barnegat are scarcely typical of those existing in the more important oyster beds, which are usually in much deeper water. On the other hand, the locality offers favorable conditions for investigations concerning the availability of many different sources of food materials for the oyster, and especially for studies on the part which may be played in the oyster's diet by food materials produced on the land or in land-locked waters, and then washed down over the oyster beds.

Besides plankton, there is suspended in the water over the oyster beds more or less organic detritus, that is, fragments of larger plants and animals and dead plankton forms; and also much inert matter, such as grains of sand and mud, the absolute and relative amounts of these materials being high after storms and low after periods of calm weather. The feeding habits of oysters make them an excellent means for separating the plankton from inert matter, the oyster having the ability to discard much sand and mud both at the gills and at the palps. The mechanism is not perfect, some food being discarded with the waste matter, and some sand, occasionally a great deal, finding its way into the stomach. LOHMANN (14) has pointed out that certain of the plankton animals (*Appendicularia*) are much more efficient collectors of nannoplankton than any net which has yet been devised. GRAN (7) reports finding abundant animal life in the Straits of Gibraltar, accompanied by an apparent scarcity of plant life, as evidenced by the net collections. Examinations of the stomachs of Salpae, however, showed that

these animals were feeding almost entirely on nannoplankton forms which were too small to be caught in the nets. The oyster displays similar ability. It is a better collector of the minute forms than any net. In summer it is nearly as efficient a collector of the larger forms, although, as NELSON (**23**) has pointed out, some of the very spiny diatoms and protozoa, and also the crustacean zoeas with their large dorsal spines, are of such a shape that it would be very difficult for an oyster to handle them. NELSON finds further that copepods, nauplii, rotifers, and worm larvae disappear from the food in cold weather, indicating that at such times the ciliary currents of the oysters are too feeble to permit them to capture the more active forms.

References to the food of the oyster are numerous, some of them being based on careful and painstaking study; others on insufficient data or mere assumptions. One of the earliest careful studies was made by DEAN (**6**), working in New York waters. He concluded that 88 per cent of the oyster's food supply is composed of diatoms, 1 per cent or less of desmids, 3 per cent of spores and particles of seaweed, and the remaining 8 per cent of animal forms. He regarded the diatoms which develop at the mouths of brackish creeks as particularly important. LOTSY (**15**) regarded diatoms as practically the only source of food. He did not believe that either animals or ground organic matter were of any importance, but suggested that in winter and early spring the reproductive cells of the larger algae might prove to be an additional source of food. MOORE (**19**) has made extensive studies of the food of the oyster. In his study of certain Texas oyster beds he made elaborate investigations of the plankton organisms, filtering the water through fine sand, separating the organisms out, and killing them in formalin. Convinced that diatoms usually constitute about 95 per cent of the oyster's food supply, he devoted himself to studying the rate of feeding and the calculation of the food value of present or potential oyster producing waters. He devised an elaborate method for removing entire contents of an oyster's digestive tract (**20**). The organisms so secured were carefully examined to determine just what forms are eaten, and the rate of feeding was ascertained by an ingenious method. Plankton collections were then made, the

principal organisms known to be eaten were counted and their average volume calculated. By multiplying the number of individuals of each species by the average volume of that species and adding the results, the total "food value" of any given area of water could be secured. MOORE'S results, while extremely valuable, are subject to certain corrections. In the first place, calculations of the comparative food value of food organisms on the basis of relative volume are of doubtful validity when different species of diatoms are compared with each other, and very largely lose their significance when diatoms are compared with peridines, or tissue fragments of larger plants, as has been demonstrated by BRANDT (quoted by GRAN 7), and by the analyses of JUDAY (9). Again, in calculating the potential value of waters where oysters had not been growing, he did not take into account the fact that the presence of oysters might materially increase the number of food organisms in such waters. Finally, he did not consider the possibility that the nannoplankton and organic detritus may constitute a significant part of the food supply.

KELLOGG (10), apparently relying on previous work, repeats the statement that almost the entire food supply is composed of diatoms. GRAVE (8) has made extensive studies on the Atlantic coast, listing thirteen organisms which he believes constitute the bulk of the food of Chesapeake Bay oysters. Of the thirteen, ten are diatoms, two peridines, and the remaining one a small green flagellate. On the basis of his data he calculated the food value of the Chesapeake waters according to MOORE'S method. PETERSEN (24), studying the European oyster, expressed doubt whether either the plankton or bottom diatoms played any considerable part in the nutrition of the oyster, and later PETERSEN and JENSEN (25) definitely announced their conclusion that the essential food of all the bottom-feeding marine invertebrates is the dust-fine detritus composing the upper layer of the bottom in the more quiet waters, and made up of dead deposited particles of animals and plants, plus inorganic materials, and in much less degree fragments of plankton organisms. *Zostera* they considered as by far the most important producer of organic matter. They found the stomachs of oysters full of this fine ooze from March to November, with only

very few organisms present, of which the commonest was *Prorocentrum micans*, a peridine. In winter they found the stomachs empty as a rule. BLEGVAD (3), associated with PETERSEN in Denmark, in a later paper is reported as concluding that the living phytoplankton is of no importance at all as food for the bottom fauna, the plankton algae not being available until they die and fall to the bottom, that is, become detritus. MITCHELL (16) studied the problem experimentally. He kept oysters in glass jars of filtered, aerated water, and fed them with finely chopped *Ulva* and protozoa. Such oysters at the end of fourteen and seventeen days respectively were richer in glycogen than oysters of the same lot analyzed when brought from the beds, indicating that the former had fed on these substances. He also reported experiments on the absorption of dissolved organic matter, referred to later. NELSON (21) was the first to point out that the growths on the shell of the oyster, including, as they do, numerous diatoms and other minute forms of life commonly found in the stomachs of the oysters, are possible additional sources of food. In a later report (23) he records a number of important observations on the food of oysters, finding that during certain seasons of the year much animal food is ingested, including protozoa, roundworms, microcrustaceae, the larvae of such forms as polychaete worms, snails, clams, oysters, and tunicates, and eggs of various sorts; in short, that any plankton form less than 0.5 mm. in length may be taken in and digested. He found very distinct seasonal variations. In late winter and early spring, filamentous diatoms are the most important food elements, with few animals. In summer, non-filamentous diatoms and many animals are taken, the latter sometimes comprising 80 per cent of the food. In late summer, eel grass and other ground plant material constitute the principal food, while in winter no animals except protozoa are taken, the food consisting almost entirely of diatoms, with some detritus. He also found that, contrary to what had been believed, the amount of suspended inert matter in the water has little or no effect on the oyster's feeding, and that even at times when there is a large amount of sand and mud in the water, the food is largely separated from it before reaching the oyster's stomach.

Two papers by ALLEN on the food of fresh-water mussels have considerable bearing on the present problem. In the earlier paper (1) he reported that the food of these animals is much more varied than had previously been supposed, small animal forms and many algae being taken, as well as diatoms. He found that the crystalline style disappeared when a mussel was starved, and reappeared when food was supplied. In his later paper (2) he showed experimentally that the nannoplankton is of at least as much importance as the net plankton, and suggested that owing to its smaller size it is probably more readily assimilated. In all his experimental work he used the crystalline style as the criterion of feeding, reporting that the style can never be regenerated except by substances which have food value. He also reports somewhat inconclusive results tending to verify his belief that the shell growths contribute considerable food.

The theory that marine animals, including oysters, may derive a substantial part of their nourishment, not by digesting organized material, but by absorbing organic substances dissolved in the water, was first advanced by PÜTTER in 1907 and stated in elaborate form in 1909 (26). PÜTTER's arguments were based on four considerations: the relatively small amount of plankton present in the ocean; the comparatively large nutrient need of the animals; the large amount of dissolved organic matter present in sea water; and the small number of plankton forms present in the stomachs of marine animals. Numerous investigators have shown that PÜTTER's estimates of the plankton are much too low, while his estimates of the nutrient needs of the animals are much too high, as are his figures for the amount of dissolved organic matter in sea water; and finally, that the data from the examinations of stomach contents are inconclusive. Of the many papers touching on these points, only a few are mentioned which have particular bearing on the present problem. DAKIN (5) showed that the copepods have maxima closely following the maxima of diatoms and peridines, and that the mass of amorphous green material commonly present in their digestive tracts in all probability represents the remains of naked nannoplankton forms, which constitute an important food source for the copepods, but which are too small to be caught by

plankton nets. WOLTEREK (28) showed that in two connected bodies of fresh water the plankton animals and plankton-consuming fish were much more abundant in the upper than in the lower, although in the latter the net phytoplankton was much more abundant. He found that the upper body contained a very rich nannoplankton flora, including numerous forms too small to be retained by a leather filter, and explained this by the fact that its waters were much richer in dissolved organic matter. Both DAKIN and WOLTEREK emphasize the greater availability of the minute forms as compared with the larger phytoplankton species. LOHMANN (13), in a critical review of PÜTTER'S theory, points out that many of the animal forms when freshly captured contain a rich food supply, while in the same forms, when preserved, only a scanty number of food organisms can be recognized, hence studies of the stomach samples, to be of complete value, should be based on collections just out of the water. MOORE, EDIE, WHITLEY, and DAKIN (18) discuss PÜTTER'S analyses in detail, and show the sources of error in his computations, concluding that his estimates of the amount of dissolved organic material are absurdly high. They also point out that such of this matter as is present is much more available to minute unicellular forms than to larger organisms. LIPSCHÜTZ (12) summarizes the objections to the theory, and emphasizes the importance of the unconsidered nannoplankton.

CHURCHILL (4) experimented on fresh-water mussels, kept in five liter aquaria, adding to the water very small amounts of protein, starch, and fats. The results were taken to indicate that under such conditions the mussels could absorb these substances, and on this basis he contends that in nature not only mussels but other aquatic animals can utilize nourishment of this sort. MITCHELL, in the earlier of the papers already referred to, made similar experimental studies on oysters, keeping them in sea water containing small amounts of dextrose, and showing that they were able to absorb this sugar and store it as glycogen. In his later paper (17) he submits additional data indicating that areas of shallow water receiving the drainage of bordering low, swampy land areas contain dissolved organic matter which is favorable to the nutrition of oysters. He found free ammonia to be slightly higher, and albumi-

noid ammonia distinctly higher in such waters than in those bordered by less swampy lands, and states that such waters are popularly preferred as fattening grounds. The conclusion that oysters make use of the dissolved organic matter does not follow from this observation, since such conditions would favor the rapid multiplication of the nannoplankton forms, and it must be regarded as extremely improbable that the oysters could compete with these forms in the utilization of such substances, although the nannoplankton would itself be rich feeding for the oysters. The conditions in the experimental work cited were so different from those obtaining in the open waters, that the results can be applied to natural conditions only with great caution and many reservations. It must be regarded not only as unproved, but as improbable, that dissolved organic substances play an appreciable part in the nutrition of the oyster.

It will be seen that three distinct sources of food have been suggested, namely, small marine organisms, especially diatoms; organic detritus; and organic substances dissolved in the water; and that various workers are still far from agreeing as to their relative importance. Many of the earlier workers were inclined to consider diatoms as practically the exclusive source of food. Later workers have come more and more to a realization of the importance of other organisms and of detritus. No American students have taken the extreme position of those European workers who hold that detritus is all important, and that living organisms constitute a relatively insignificant part of the oyster's diet. Inasmuch as the European oyster is specifically distinct from the American, it is possible that European and American results are not strictly comparable. There is no question but that the American oyster does consume detritus, and if this constituent plays anything like the part in its nutrition as it seems, on excellent evidence, to play in the nutrition of its European relative, it is of great practical importance to determine that fact.

As has been pointed out, the evidence for the direct utilization of dissolved organic substances by the oyster is scanty and inconclusive, and does not indicate that under ordinary conditions such nutrition plays any significant part. Since the facilities and

equipment available at Barnegat did not permit the carrying on of properly checked experimental work on this phase of the question, this possibility was disregarded in planning the field work. Attention was focused on the two other possible sources: (1) small organisms, and especially the phytoplankton forms naturally occurring in the waters over the oyster beds; and (2), detritus, including in that term ground *Zostera*, *Ulva*, *Enteromorpha*, and other of the larger marine plants, as well as fragments of land plants, pollen grains, and fungus spores washed from the land. The term plankton is used to include any of the minute organisms found in the oysters' stomachs or in the waters over the oyster beds. In such a locality as Barnegat the true pelagic forms are much less important than the bottom or littoral forms, but this is true in greater or less degree of all oyster waters. The arbitrary division on the basis of size into net plankton and nannoplankton is familiar, the former including such organisms as are retained by a silk bolting cloth with two hundred meshes to the inch, the latter including the organisms able to pass through such a net. Most of the forms with any dimension of over 25 μ are retained by the net. Under certain circumstances it is more important to distinguish between organisms normally present in the waters over the oyster beds, and organisms originating elsewhere and washed into such waters.

The field studies may conveniently be treated under three heads: (1) regular examination of the stomach contents of oysters; (2) experiments in feeding oysters various materials; (3) studies on the shell growths of oysters.

Examination of stomach contents

Most of the stomach samples were taken from oysters kept in floats near the laboratory, but some examinations from other nearby localities were made. This limitation was necessary because, as explained later, examination of the fresh contents within a few minutes after the animal is removed from the water is the only way to obtain trustworthy results. In taking the samples, the method described by NELSON (**23**) was used. The palps were washed with clean salt water and a small portion of the stomach contents was withdrawn by means of an ordinary rubber bulb pipette. No attempt was made to empty the stomachs. Notes

were at once made on the appearance of the sample, the presence or absence of a crystalline style in the individual, and its color and consistency if present. A small portion of the sample was then examined immediately and careful notes made concerning the organisms present while they were still alive. The remainder of the sample was then preserved in formalin and put aside for future study. When this was undertaken, it brought out the important fact that such preserved samples appeared on analysis to be strikingly different from the same sample when freshly taken. The diatoms, armored peridines, small algae and algal fragments, fungus spores, and pollen were in nearly all instances satisfactorily preserved. On the other hand, the naked peridines and flagellates, often an extremely important part of the sample as seen in the fresh mounts, had entirely disappeared, apparently merged with the mucus and other amorphous material always present in small amount. While this statement applies particularly to stomach samples, it is also true in less degree of plankton samples and shell growths, and of material kept in other preservatives than formalin. This is in accord with LOHMANN'S (14) observations, and with the experience of KOFOID and SWEZY (11) as to the extreme sensitiveness of the naked peridines, and emphasizes the fact that studies on the food of the oyster based on preserved material must be regarded as incomplete. Such conclusions as are here offered, therefore, are based very largely on examinations of fresh samples, necessarily more or less hurried, and are only roughly quantitative. Nevertheless, they clearly indicate certain untouched phases of the problem that demand fuller investigation. These are as follows:

1. EXTREME VARIABILITY OF FOOD SUPPLY.—NELSON (23) emphasized the great seasonal fluctuations in the kind of food taken, but under the conditions prevailing at Barnegat, marked fluctuations may be observed within a few hours. Following strong northeast winds, with high tides, the stomachs of the oysters contain numerous organisms characteristic of the waters of the open bay, conspicuous among which are large diatoms belonging to *Coscinodiscus*, *Melosira*, *Pleurosigma*, and *Navicula*. Following hard rains, these forms almost disappear, and are replaced by small diatoms developed along the shores of the more brackish waters of

the creek, of which the most important is *Schizonema ramosissima*, together with peridines and small flagellates, as well as by the organisms mentioned later which develop in the salt ponds. Occasionally certain peridines, especially a species of *Glenodinium* apparently the same as that noted by GRAVE (**8**) as abundant in oysters' stomachs in Chesapeake Bay, constitute the bulk of the stomach contents. At other times the stomachs are filled almost exclusively with nannoplankton (small diatoms, flagellates, zoöspores of algae, etc.), with only an occasional larger organism.

2. IMPORTANCE OF NANNOPLANKTON.—This has already been suggested in the preceding paragraph, but should be emphasized. In numerous collections the nannoplankton organisms occurred in such abundance that in spite of their small individual size, it would be conservative to estimate that they comprised over 90 per cent of the food present, while in a smaller number of collections it was difficult to find any larger species in a given amount. This was more particularly the case during the very rainy summer of 1922, when the proportion of nannoplankton was noticeably greater than during the drier summer of 1921.

3. IMPORTANCE OF ORGANISMS WASHED FROM BRACKISH PONDS INTO WATERS OF OYSTER BEDS.—The area of salt marshes bordering the southern part of Barnegat Bay is approximately equal to the area of the adjoining part of the bay. Scattered about these marshes are the shallow brackish ponds previously described. The plankton occurring in these ponds, as has been pointed out, is extremely rich, even as compared with the rich waters of the lagoon, and is quite distinct in its composition. Among the characteristic forms may be mentioned certain species of *Exuviella*, *Amphora*, *Nitzschia*, *Surirella*, and *Euglena*, all of which occur sparingly or not at all in the stomach samples except after rains, when they become very abundant. During the very rainy summer of 1922 *Euglena* was especially abundant. This organism was regarded as belonging to the nannoplankton, since in spite of its moderate size, its extreme flexibility permitted it to pass readily through bolting cloth. Pollen grains and fungus spores are frequently conspicuous in the stomach samples after rains, but cannot be regarded as of importance.

Feeding experiments

During the summer of 1921 and the early part of the summer of 1922 feeding experiments were conducted in rectangular wooden boxes having inside dimensions of 4×6×12 inches, with the ends of bolting cloth. A removable slab in one side, which could be tightly sealed with absorbent cotton between cheesecloth, permitted the insertion of an oyster, while a hole in the top, closed by a cork, permitted any desired food material to be introduced. This method is a modification of that used by ALLEN (2). The oysters to be used were carefully scrubbed to remove shell growths and then placed for about two hours, first in a small quantity of clean sea water, then in filtered water, to permit them to empty their digestive tracts. The shells of the first oysters used were scrubbed with a two per cent solution of formalin, but in some cases this seemed to interfere with the functioning of the animal, and in one case was presumably the cause of the death of an oyster in one of the boxes, so the use of formalin was discontinued. Preliminary tests made with two, three, and four oysters in a box at one time showed that it was inadvisable to keep more than one oyster at a time in boxes of the size used. The boxes, with the oysters in them were placed in a float arranged so that the current would flow through the ends of the boxes. After using, the boxes were carefully scrubbed each time with fresh water, and the bolting cloth swabbed with moistened absorbent cotton.[1]

In examining an oyster after an experiment, not only the stomach contents, but the presence or absence of a crystalline style, and the color and consistency of the latter, if present, were used to determine the results. ALLEN (2) used the style as a feeding test in his work with fresh water mussels. NELSON (22) states that "the style of an oyster may be dissolved within an hour [after feeding stops], often in much less time. Furthermore, a new style may be built up within fifteen minutes from the time the animal begins active feeding." It was assumed, therefore, that the presence of a

[1] The handling and subsequent motion, due to the action of the waves on the float in which the boxes were kept, did not interfere with spawning. In several instances the stomachs of oysters under experiment in the boxes were full of eggs, and in cases where male and female individuals had been confined in the same box, of developing oyster larvae.

well developed style could be taken as an indication that the animal had been feeding vigorously, and that if the style were flaccid it might mean either that the food supply was scanty or that the animal had stopped feeding. In order to make sure that the oysters subjected to treatment in filtered water would lose their styles, on a number of occasions an extra oyster was placed with the lot, usually of four, to be used in an experiment. When opened after an hour or two in filtered water, these extra oysters, in the earlier trials, had invariably lost their styles. On this basis experiments were conducted, letting the animals feed on organisms that could pass through the bolting cloth, that is, on nannoplankton alone, and also adding to this shell growths, ground seaweed, ground eel-grass, and ground marsh grass.

1. FEEDING NANNOPLANKTON.—After discarding all experiments in which the box leaked or in which spawning females ate their own eggs, there remained twenty-nine tests, in twenty-five of which a fully developed style was formed, differing only in its lighter color (hyaline or pale greenish or yellowish) from the styles formed at the same time by oysters feeding naturally. In every such case the stomach contents consisted almost wholly of nannoplankton, the exceptions being a few forms on the border line, that is to say, somewhat over 25 μ in one dimension. These were either flexible, as *Euglena*, or long and slender diatoms such as small species of *Nitzschia*, and not as a rule in significant numbers. In two instances *Prorocentrum micans* (averaging 40×24 μ)was abundant. The stomachs were never as full as in the case of oysters feeding normally.

2. FEEDING SHELL GROWTHS.—Three oysters were scrubbed and put into boxes into each of which the shell growth scrubbed from another oyster was introduced When examined these three oysters had developed large dark colored styles, and the stomach samples were very similar to those of other oysters feeding outside of the boxes. In another trial two oysters with normal green shell growths were each put into a box without being scrubbed. One of the oysters when collected had no style and nothing in its stomach, and had evidently not been feeding. The other had a fully developed style and a fairly full stomach, the contents, however, consisting mainly of nannoplankton, indicating that few of the shell

growth organisms had reached the stomach. This would seem to indicate nothing more than that the current inside of the boxes was too weak to sweep the organisms from the shells, and not that the shell growths are unimportant sources of food when the oysters are exposed to the normal movements of the water.

3. FEEDING GROUND MARINE PLANTS.—Trials were made with *Ulva*, *Enteromorpha*, fresh *Zostera*, and partly decayed *Zostera*, all carefully washed in several changes of clean sea water, then in filtered sea water. They were then ground in a mortar with clean beach sand and filtered water, and strained through scrim cloth, forming thick "soups" similar to those used by MITCHELL (16). It was not possible to get these plants absolutely clean, but the *Enteromorpha* "soup" prepared in this way contained surprisingly few diatoms, and almost no other foreign matter. The *Ulva* "soup" was much less free from diatoms, and both the fresh and the partly decayed *Zostera* preparations contained a great many, consequently *Enteromorpha* was used more than the others. Even the *Zostera* suspensions, however, contained a large proportion of recognizable *Zostera* fragments. Five of the seven trials with *Enteromorpha* gave positive results, the stomach samples including much recognizable ground algal material and comparatively few diatoms or other organisms. The styles of these five oysters were a striking bright grass green in color. Two trials were made with *Ulva*, and positive results were secured in both cases, the styles in these instances being of a similar bright green. As was expected, there were more diatoms present in the stomachs of these oysters than when the *Enteromorpha* was fed. One trial was made with fresh *Zostera* and one with partly decayed *Zostera*. In both cases a clear yellow brown style was produced, and the stomachs contained, besides numerous *Zostera* fragments, many diatoms and other organisms that had been fed with the *Zostera*. In all these trials, of course, nannoplankton forms were also present.

4. FEEDING A LAND PLANT.—The common marsh grass of the flats adjoining the oyster beds, *Spartina glabra* var. *pilosa*, was selected, and was prepared in the same way as the marine plants, except that it was not necessary to wash it. The suspension was fed to three oysters in three boxes. All were collected under

favorable conditions of tide and salinity, and when oysters in the floats nearby were actively feeding. The stomachs were all empty and no styles were present. At the bottoms of the mantles, however, conspicuous masses of the ground *Spartina* were imbedded in mucus, preparatory to being discharged, indicating that this material had been definitely rejected.

The preceding experiments were taken to indicate that all these materials except the *Spartina* were utilized as food by the oyster. Early in the course of the 1922 work, however, certain irregularities appearing in the checks led to the suspicion that the renewal of the style might be due to other factors than feeding, and a number of experiments were made to determine this point. NELSON'S statement concerning the disappearance of the style when the animal is out of water was in general verified. In one trial, made on a cool cloudy day, two oysters of four taken out of the water at the same time had pale but firm styles when opened at the end of an hour. The other two were opened at the end of two hours, and had then completely lost their styles. It was found that in general oysters kept in filtered water lost their styles after an hour or so, but when a large number were treated in this way there proved to be exceptions to the rule. After three or four hours in such water, the styles not infrequently were more numerous than after an hour or two, suggesting that they had been regained. The results of a large number of trials are too irregular to tabulate, but they indicate that while the regeneration of the style is usually a response to the presence of food, it may be brought about by the mere act of siphoning in the complete absence of food. If the animals are roughly handled, they do not siphon and the styles disappear. If they are handled gently, the style is frequently present after an hour or more in carefully filtered water. If they are left in such water, in an aquarium, so long that the oxygen is largely exhausted, they do not siphon and the styles usually (not always) disappear. If the same water is aerated, siphoning may begin again and the styles may be partially or even completely renewed.

Obviously, these results make it impossible to regard regeneration of the style as a final criterion of feeding. It remains true,

however, that the style is of very considerable value as an indication of feeding, not only on the basis of its presence or absence, but also on that of its varying color and consistency, following exposure of the animal to different conditions. On this basis an additional series of feeding experiments was conducted, using five gallon aquarium jars about two-thirds filled with filtered water, and containing from five to ten carefully scrubbed oysters at a time. Various means of getting the water free from nannoplankton were tried. The water was filtered through cloth and absorbent cotton as well as through filter paper; it was filtered, then heated to 70° C. to kill all organisms, then let stand over night to settle, siphoned off, and refiltered, letting it fall through air to aerate it. Best results were secured, however, by filtering the water through cheesecloth and absorbent cotton, letting the oysters stand in it for a few hours, then refiltering. After this process had been repeated several times, practically all of the nannoplankton forms had been removed by the oysters themselves, and the small amount of feces accumulating was in the form of strings of colorless mucus. When this stage was reached, the same food materials were added to the water as had been used in the box experiments. In addition, a small green alga with globose cells 2–3 μ in diameter was fed, representing a nannoplankton form. This species was used because it could be secured in pure culture and multiplied very rapidly in flasks of sterilized sea water to which small amounts of nutrient salts were added. Before starting an experiment, the oysters were kept in the same water for from two to five days, and the water was refiltered and aerated at least half a dozen times. The results are summarized in table I. The figures in parentheses indicate the number of animals in which the styles were poorly developed or very flaccid. All doubtful results, including cases in which eggs were liberated into the water, have been excluded.

It will be noted that in eighteen out of twenty cases in which food was found in the stomachs a style was present, while of fifty-one oysters in which no food was found in the stomachs, only sixteen had styles and nine of these styles were poorly developed. In the case of two of the three checks in which both food and a style were found, the food consisted of reworked feces. In the remaining case

it consisted of numerous extremely small (1.5–2 μ) and nearly transparent globose bodies, apparently blue-green algae. These were so difficult to see that they were not noticed in a first examination, but only upon more careful study under a higher power. Similar forms may have escaped notice in some of the ten checks in which styles were present but no food found. In the case of the two checks in which food was reported present, but no styles, the food consisted mainly of large diatoms of the sort frequently found in oysters freshly removed from the water. They had evidently been carried into the jars within the shells of these animals, which had remained practically dormant. The smaller forms, however, had nearly all disappeared from these stomachs.

TABLE I

Oysters	Style present, food in stomachs	Style lacking, food in stomachs	Style present, no food in stomachs	Style lacking, no food in stomachs	Totals
Fed shell growths	2	.....	.....	.....	2
Fed Enteromorpha	3	.....	.....	1	4
Fed Ulva	2	.....	.....	2	4
Fed Zostera	2	.....	2 (2)	1	5
Fed Spartina	2	.....	3 (3)*	3†	8
Fed small green alga	4 (1)	.....	1	3	8
Checks	3	2	10 (4)	25	40
Totals	18 (1)	2	16 (9)	35	71

* *Spartina* rejected at base of mantle by one of these animals.
† Two did not feed at all; one rejected the *Spartina*.

In the eight trials with ground *Spartina*, two of the oysters fed and developed large, firm, green styles. Of the remaining six oysters, four showed no evidence of having taken in this material, although two of the four had poorly developed styles. Both of the remaining two oysters rejected the *Spartina*, as evidenced by masses of the ground material imbedded in mucus at the base of the mantle. One of these oysters had a flaccid style, the other had no style. In comparing these results with those in which the same material was fed in the boxes, it should be remembered that the oysters in the jars had been starved and hence may possibly have taken food that they would not ordinarily use. The indication is that the *Spartina* may be utilized, but under ordinary circumstances would

not be. The other results seem to agree with the box experiments, and to require no special comment.

Studies on shell growths

1. COMPOSITION OF SHELL GROWTHS.—An oyster freshly removed from the water has the shell covered with a slimy dark, greenish brown scum which easily rubs off. Microscopic examination shows this to be a more or less gelatinous mass in which are living numerous diatoms, protozoans, round worms, and other small organisms. The diatoms are very largely the same species as those present in the stomachs of the oysters on which the growths occur. In addition to this growth certain of the larger algae are nearly always present in some amount, and frequently grow very luxuriantly, serving as a substratum for numerous diatoms, especially species of *Licmophora*, *Melosira*, *Rhabdonema*, *Acnanthes*, and *Synedra*, all of which are of common occurrence in the stomachs. NELSON (21) notes that these bearded or "whiskered" oysters are regarded by practical oystermen as certain to be fat.

2. RATE OF ACCUMULATION OF SHELL GROWTH.—In order to test the rapidity with which the shell growths can accumulate, twelve oysters of uniform size were carefully scrubbed and put on a float. One of these oysters was removed each day for ten days, one on the twelfth, and one on the fifteenth day. The shell growths were carefully scrubbed off into clean sea water, an equal amount of 10 per cent formalin added, and the whole allowed to stand over night. Next morning the bulk of the clear liquid was decanted from the material which had settled, the remainder being preserved in a vial. Fig. 2 shows graphically the rate of accumulation for the first ten days. Microscopic examination disclosed a striking difference between the earlier and later accumulations. The accumulation of the first day was composed largely of mucus and amorphous material discharged from the lower edge of the mantle, to which some of the sediment suspended in the water had become attached. Living organisms were very few. The proportion of organisms to inert matter showed a steady increase until about the tenth day. The figures show graphically the comparison between the shell accumulations of the first day (fig. 3) and those of the tenth day

(fig. 4). There was little apparent difference in the microscopic appearance of the collections of the tenth and fifteenth days.

3. METHOD OF SHELL ACCUMULATION.—Observation of oysters in aquaria as well as in the floats showed that the oyster itself has an important part in building up these shell growths. The detritus, sand, and other material strained out of the water by the gills, but rejected as food by these organs or by the palps, is imbedded in mucus and carried to the bottom of the mantle, eventually to be discharged. These mucous discharges tend to cling to the shell

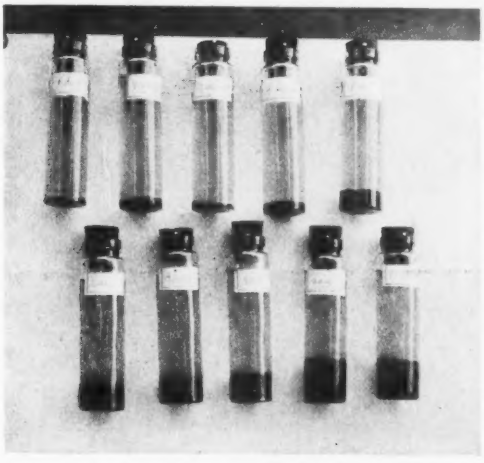
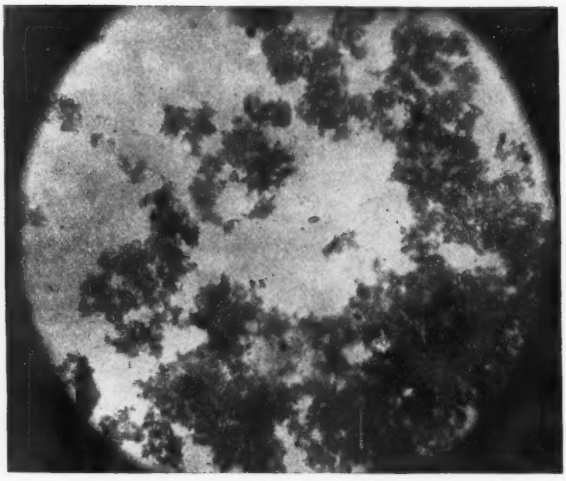


FIG. 2.—Accumulations on shells of ten oysters of approximately same size, first to tenth days.

and to form a gelatinous coating in which the diatoms and other organisms rejected with them, owing to the imperfect separation of food and waste by the animal, find a favorable medium for growth. Strings of the oyster's feces as well as particles suspended in the water adhere to the slime, and soon there is a rich growth of microorganisms on the shell. Thus the oyster not only plants a garden on its shell but also fertilizes it.

4. COMPARISON BETWEEN RATE OF ACCUMULATION ON SHELLS OF LIVING OYSTERS AND ON EMPTY OYSTER SHELLS.—If the foregoing account of the nature of the shell growths is correct, it follows that



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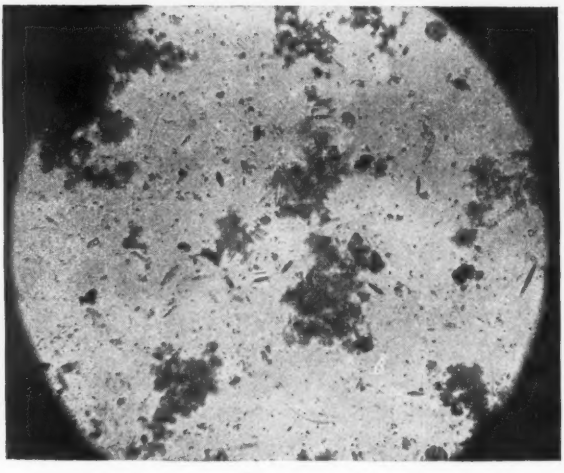

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FIGS. 3, 4.—Fig. 3, accumulations of one day (note almost entire absence of organisms); fig. 4, accumulations of ten days (note relative abundance of organisms as compared with inert matter).

the rate of accumulation should be more rapid on the shells of living oysters than on empty shells, since the chances are in favor of the mucous discharge clinging to the shell of the animal which formed it, rather than to another shell. In order to test this point, five medium sized oysters were selected, and also five right and five left shells, picked for their resemblance in shape to the living oysters and slightly larger than these. The oysters and the shells were placed in floats side by side, the empty shells lying in the same positions as the corresponding shells of the living oysters. Three tests were made in as many localities under greatly varying current and weather conditions, and lasting for four, seven, and eleven days respectively. At the end of each test the shells of the live oysters were carefully scrubbed, also the outer sides of the empty shells, and the accumulations collected in the manner previously described. In every case the accumulation on the live oysters was much greater than that on the empty shells (fig. 5), and the material from the living oysters was markedly richer in microorganisms than that from the empty shells.

FIG. 5.—Accumulations of eleven days on five live oysters (right) and on five pairs of similarly shaped but slightly larger shells (left).

To test this point further, a number of empty paired shells still connected by their hinges were collected from the water and filled with concrete. The concrete set very quickly, and it was necessary to keep the shells out of water only about four hours. Five of these concrete oysters were matched with five similarly shaped but slightly smaller live oysters (fig. 6), and put side by side on floats. Four tests were made, lasting two, ten, twelve, and fourteen days respectively. In the two day test (fig. 7) the accumulations on the live oysters amounted to about two and one-half times as much as those on the concrete oysters; in the fourteen day test (fig. 8) the amount was almost the same for both; the other two tests were intermediate. After a certain amount of the shell

growths accumulates, the material seems to be washed off by the currents almost as fast as it is formed. Under the conditions

FIG. 6.—Five living oysters (upper row) and five similarly shaped, concrete-filled shell pairs (lower row), used in experiments to determine relative rapidity of accumulation.

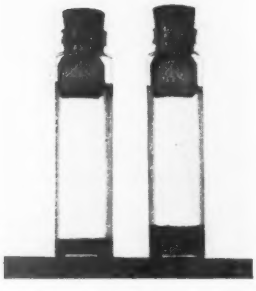

FIG. 7

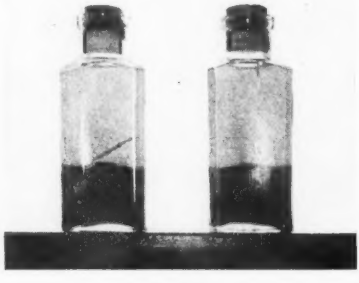

FIG. 8

FIGS. 7, 8.—Fig. 7, Accumulations of two days on living oysters (right) and on concrete oysters (left); fig. 8, accumulations of fourteen days: living oysters (right), concrete oysters (left); amount of accumulations approximately equal.

studied, this stage appears to be reached in from two to three weeks. The accumulations on the live oysters were always richer in micro-

organisms than those on the concrete oysters. A number of nannoplankton forms always failed to settle and were lost when the water above the sediment was decanted. Such organisms were always much more abundant in the washings from the live oysters than in those from the concrete oysters (fig. 9). It is obvious, therefore, that the living oysters must contribute more to the general food supply than empty shells or other inert objects in the water.

FIG. 9.—Accumulations of twelve days: living oysters (right), concrete oysters (left); note greater clearness of liquid over accumulations from concrete oysters.

Summary

1. Three sources of nutrition have been reported as available to the oyster: small living organisms, organic detritus, and substances of organic origin dissolved in the water. The first two of these sources are considered in this paper.

2. Of the living organisms, diatoms seem to be of less importance than has previously been indicated, although undoubtedly very important. Nannoplankton forms, especially small flagellates and peridines, may under some conditions constitute the bulk of the food. The occurrence of the various food organisms is subject to sudden local as well as seasonal fluctuations.

3. Studies on the food of the oyster based on preserved collections are inconclusive, since many of the smaller and more delicate organisms are destroyed by the preservative agents.

4. Shallow land-locked waters adjoining the oyster beds are important sources of numerous food organisms.

5. The development of a crystalline style is usually correlated with the taking of food, but this structure may appear in the complete absence of food, possibly as a response to the act of siphoning.

6. The color and consistency of the style may be used, with reservation, to indicate the amount and to some extent the kind of

food taken. The presence of the style, however, is not in itself evidence that food has been taken.

7. There is some indication of rejection by the oyster of finely ground particles of a land plant, the marsh grass *Spartina glabra* var. *pilosa*.

8. The growths on the shells of oysters are largely due in the first instance to the excretions of the animals themselves; they accumulate more rapidly on the shells of living oysters than on empty shells. The first accumulations are markedly poor in living organisms suitable for use as food by the oyster, the later accumulations rapidly becoming richer.

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CELL STRUCTURE AND CELL DIVISION IN THE CYANOPHYCEAE

ARTHUR W. HAUPT

(WITH PLATE VII)

Introduction

The Cyanophyceae comprise the simplest group of autotrophic Thallophytes, and exhibit in cell structure, reproduction, and organization of the plant body a combination of extremely primitive features. Not only on account of their structural simplicity, but also because of their great age and their power of withstanding extreme environmental conditions, these plants are of considerable evolutionary interest. Their affinities are with the bacteria rather than with the higher groups of algae; yet many evolutionary tendencies are seen in the Cyanophyceae along which the more highly organized algae have probably passed in the course of their evolution.

To the cytologist who seeks in the lowest plants a basis for an understanding of the complex cell structure and cell physiology of the higher plants, the Cyanophyceae are of particular interest, for correlated with a primitive cell structure is a comparatively simple mechanism of cell division. The cytology of the Cyanophyceae has been studied by about fifty investigators, and a considerable accumulation of literature has resulted. In spite of this extensive investigation, both the results obtained and the manner of their interpretation have been very diverse. Probably no problem in plant cytology has been a matter of greater difference of opinion than the structure and division of the cell of the Cyanophyceae. Much of this lack of agreement, particularly among the older investigators, has been due to faulty technique.

Material and methods

The present study was undertaken in an attempt to apply to this problem the same careful methods of technique and study as are used in modern cytological investigations on the higher

plants. Of the genera used in the investigation, including *Gloeocapsa*, *Coelosphaerium*, *Oscillatoria*, *Phormidium*, *Lyngbya*, *Nostoc*, *Anabaena*, *Tolypothrix*, and *Rivularia*, the genus *Anabaena* was selected as the most favorable for intensive study, as the same methods of technique used on all of the available forms yielded the best results with *Anabaena*.

In August 1918 *Anabaena circinalis* Rabenhorst (*A. gigantea* Wood) was found by the writer as *Wasserblühte* around the edges of a lake at Mercer, Wisconsin. At the time of collection this species seemed to be particularly favorable for cytological investigation, for with only ordinary methods of technique the details of structure were seen with a remarkable degree of clearness. A comparative study of other species of *Anabaena* was made, but in none of them was the structural detail as clearly seen as in *A. circinalis*. Consequently this species has been made the basis of the present investigation. It may be taken as a typical representative of the order Hormogoneae. In addition, it seemed advisable to study intensively a member of the lower of the two orders of Cyanophyceae, the Coccogoneae, and *Gloeocapsa aeruginosa* (Carm.) Kg. was used. Its structure, however, cannot be as clearly demonstrated as that of *Anabaena*.

The most satisfactory and conveniently used killing and fixing reagent for the material studied was found to be 5 per cent formalin. Chromo-acetic acid of 0.25 per cent strength was used both with and without a small quantity of 1 per cent osmic acid, but no better results were obtained than with formalin. A great number of preparations were made by merely allowing a drop of formalin material to dry on a slide. After staining, dehydrating, clearing, and mounting in balsam, very good views of the cell contents were obtained. When this method could not be used, preparations were made by the Venetian turpentine method. For critical study, however, microtome sections were vastly superior to all other preparations. Such forms as *Phormidium*, *Nostoc*, and *Rivularia* were easily handled by the usual methods of paraffin imbedding. In preparing *Anabaena* and *Gloeocapsa* for sectioning, the formalin material was washed in several changes of water, and then put through a very gradual series of alcohols and xylols. This was

accomplished by placing the material in a tall shell vial, allowing it to settle, and removing each change of liquid with a pipette. In this way very little material was lost.

All material imbedded in paraffin was cut to a thickness of 2μ. The most successful staining was done with Heidenhain's iron-alum haematoxylin. Safranin gentian-violet was found to be a good combination for differentiating the granules, but it does not stain with the same distinctness as the haematoxylin. In some cases erythrosin, orange G, or Bismarck brown were used as light counterstains with the haematoxylin.

The crude methods of handling the Cyanophyceae described by some authors, such as killing in strong iodine solutions, allowing material to dry, transferring material to 95 per cent alcohol directly from water, and many others, although perhaps not causing perceptible plasmolysis, certainly do not reveal the cell structure with the brilliancy of refined technique, even though sections are not made.

The writer is greatly indebted to Professor JOSEPHINE E. TILDEN of the University of Minnesota for determining all of the species used in the investigation.

Historical summary

The earlier students of the cytology of the Cyanophyceae were primarily interested in whether or not the cell contains a nucleus. The earliest paper in which this question was discussed is that of SCHMITZ (**10**). From a study of *Gloeocapsa*, *Anabaena*, and *Oscillatoria*, he showed that in living cells an outer colored portion (cortex) can be distinguished from a central colorless region (central body). Staining with haematoxylin revealed to SCHMITZ the form of the latter, which he called a nucleus dividing by simple constriction. He observed that the cells also contain large round globules or *Schleimkugeln* confined to the cortex. In his later papers (**11**, **12**) SCHMITZ underwent a change of opinion regarding the nature of the central body. After studying a great many species, he concluded that it is composed of a ground mass in which microsomes are irregularly distributed, and that it does not constitute a true nucleus.

The older literature has been well discussed by most of the recent writers, and it is not expedient to present here a detailed

account of all of the cytological investigations on the Cyanophyceae. It will suffice to summarize the work and opinions of the principal recent investigators.

FISCHER'S (2) study was based largely on microtome sections, chiefly of *Tolypothrix*, *Hapalosiphon*, and *Oscillatoria*. His theory that differential staining is indicative of physical, and not chemical composition, largely influenced his interpretations. Like previous investigators, FISCHER sees in living cells an outer colored *Rindenschicht* and an inner colorless *Centralkörper*, but no layer of colorless protoplasm outside of the cortex. Its existence is inferred, however, by the presence of minute granules along the cross walls. The green cortex is considered to be a true chromatophore, and hence an independent organ of the cell. FISCHER was able to isolate the chromatophores of the Cyanophyceae and other plants by a treatment with hydrofluoric acid which destroys all of the other cell contents.

Granules representing reserve food products are invariably present in the central body and in the narrow zone along the cross walls, but rarely in the chromatophore. FISCHER considers it impossible to distinguish between the granules on the basis of their reactions to stains or microchemical tests, and to determine whether the reserve food is carbohydrate or protein. Different staining capacities are the result of different physical characteristics. In addition to granules and coarser masses within the central body, there occurs a ground substance consisting of vacuolated protoplasm. FISCHER considers this to be merely that part of the protoplast which is inclosed by the chromatophore and serves to store up reserve food. "Ein selbständiges Organ der Cyanophyceenzelle ist demnach der Centralkörper nicht." He concludes that the cell contains neither a nucleus nor a nucleus-like organ, and that the central body is not a phylogenetic forerunner of the nucleus of the higher plants.

HEGLER (6) studied chiefly *Anabaena*, but also *Oscillatoria* and *Merismopedia*. His study was based on sectioned material to only a very slight extent. He considers that a nucleus is present, composed of chromatin granules imbedded in a ground substance and lacking a nuclear membrane and a nucleolus. The chromatin

nature of these granules is shown by their reactions to stains and to digestive fluids; and by their behavior in cell division, in which the granules fuse to form chromosomes which separate, strands of spindle fibers appearing between them. The new cell wall appears as a centripetally growing ring after nuclear division is entirely or almost completed.

The pigment is found in minute granules closely crowded together in the cortex. Chlorophyll and phycocyanin are blended together in the same granules. HEGLER considers them to be minute chromatophores and calls them "cyanoplasts." He finds that glycogen is the first visible product of photosynthesis, and that starch is not formed. In addition to the cyanoplasts, the peripheral region contains albuminous crystalloids and slime vacuoles; these never occur in the central region. The albuminous crystalloids (the "cyanophycin granules" of other investigators) represent reserve food, and are especially abundant in heterocysts and resting cells, being often wholly absent from rapidly growing vegetative cells. The slime globules are also albuminous.

KOHL (7) studied unsectioned material chiefly of *Tolypothrix*, *Nostoc*, and *Anabaena*. He describes a nucleus consisting of a ground mass in which is imbedded a definite chromatic substance and a greater or less number of *Zentralkörner*. These central granules are confined exclusively to the nucleus, which is rarely free of them. They are composed of an albuminous slime representing reserve food. *Tolypothrix* has many large central granules, *Anabaena* many small ones, and *Nostoc* none at all. The nucleus lacks a nuclear membrane and a nucleolus. In the resting condition fine radiations extend from the nucleus to the cell wall. Central granules may be imbedded therein, and thus appear to be located in the peripheral cytoplasm. The nucleus divides mitotically, and is rarely in a resting condition. Preliminary to division the chromatin increases in amount, and threads of a nuclear network, previously barely visible, become conspicuous and form a spireme. This breaks up into a definite number of chromosomes (6 in *Tolypothrix lanata*), which lie in the cell parallel with the long axis of the filament. The cell wall then grows inward and the chromosomes undergo a transverse segmentation, finally reforming the spireme.

During nuclear division the central granules are irregularly divided between the daughter nuclei.

The peripheral region of the protoplast constitutes the cytoplasm. It contains many minute spherical chromatophores, cyanophycin granules, fat droplets, glycogen, and vacuoles. The chromatophores contain chlorophyll, carotin, and phycocyanin. They are regularly in contact with each other, giving the cytoplasm an alveolar appearance. Glycogen is the first visible product of photosynthesis; starch cannot be demonstrated. Glycogen does not seem to lie in the chromatophores, but "im Cytoplasma unsichtbare Vacuolen zu erfüllen." The cyanophycin granules never occur in the nucleus. They are albuminous crystalloids representing reserve food, and are most abundant in resting cells and least abundant in young cells and dividing cells. Vacuoles of two kinds occur, cell sap vacuoles and gas vacuoles. The former are rare in normal vegetative cells, but appear in old cells; the latter are seen in *Wasserblühte* forms. Vacuoles always occur outside of the nucleus.

ZACHARIAS (13), one of the earlier investigators who had carried on extensive microchemical and cytological studies on the Cyanophyceae, objects to the conclusions of HEGLER and KOHL. Upon examination of their own preparations loaned to him, he concludes that no chromosomes are formed, and regards Kohl's "chromatin granules" and small *Zentralkörner* as probably identical. "Die stärker gefärbten, mannifach gestalteten Teile der Zentralkörper, wie sie die Präparate und die Figuren der Autoren zeigen, halte ich nicht für Chromosomen, sondern für . . . . Vorsprünge, Leisten, etc. der Zentralkörper."

In FISCHER's later paper (3) many of his earlier conclusions are restated, but some of them are revised. Contrary to the views of HEGLER and KOHL, he continues to regard the green peripheral region of the cell as a single large chromatophore. In it is formed glycogen, a surplus passing into the central body where it is stored. In large-celled species the central body holds a considerable quantity of unchanged glycogen, but in the smaller forms it must be condensed. Accordingly it is transformed into another carbohydrate, which FISCHER calls "anabaenin." The anabaenin forms either

central granules or "pseudomitotic coils." The latter are chromosome-like bodies. Anabaenin stains weakly and not at all like chromatin with many of the standard nuclear stains, iron-alum haematoxylin being an exception. During cell division the pseudomitotic masses of anabaenin undergo transformations resembling true karyokinesis. There is an equal distribution of the reserve food material to the daughter cells.

Cyanophycin granules are found predominantly in the chromatophore, but occasionally occur in the central body. They are albuminous and assume a crystalloidal form when large. FISCHER continues to regard the central body as cytoplasm filled with reserve food, and thus denies its nuclear nature. It contains no chromatin; however, he considers the "carbohydrate mitosis" of the Cyanophyceae to represent the phylogenetic forerunner of a true nuclear mitosis. The necessity of converting the surplus food into a permanent condensed form which could be equally divided between the daughter cells may have been followed by an excess formation of a protein product. This gradually came to have new functions, as the bearer of hereditary characters, and finally an exact mitotic mechanism developed.

OLIVE (8) studied sectioned material of *Oscillatoria*, *Phormidium*, *Calothrix*, *Nostoc*, *Gloeocapsa*, and *Cylindrospermum*. He describes the central body as consisting of minute chromatin granules inclosed by a dense fibrous achromatic network, and constituting a true nucleus. The nucleus divides by mitosis and does not pass into a resting stage, except in resting cells and young heterocysts, where a nuclear membrane is formed. OLIVE regards each chromatin granule as a single chromosome, and finds that their number is constant for each species.

In the preliminary stages of mitosis the chromatin granules form a spireme which can be seen in "favorable cases," and the achromatic portion of the nucleus constitutes a spindle connecting the chromosomes and leading from them to the cross walls. The spireme consists of a convoluted linin thread, along which the chromatin granules are scattered. The spireme splits longitudinally without undergoing a transverse segmentation, and the granules separate, a central spindle being formed. The new cell wall develops cen-

tripetally before the division of the nucleus is completed. It is of interest to note that FISCHER (3) and ZACHARIAS (14) agree that the "spindle fibers" described by OLIVE are only radiations from the central body, and that his chromosomes are central granules. The cytoplasm contains slime globules and cyanophycin granules, both representing reserve food. OLIVE agrees with FISCHER that the peripheral region constitutes a single large chromatophore in which the coloring matter is diffused. He sees no minute chromatophores.

PHILLIPS' investigation (9) was based almost entirely on unsectioned material, chiefly of *Oscillatoria*, *Nostoc*, and *Cylindrospermum*. The central body is regarded as a true nucleus, in the resting condition consisting of chromatin "aggregated in hollow vesicles," and imbedded in a finely granular ground substance. There is no nuclear membrane. PHILLIPS considers these hollow vesicles to be the same as the slime globules of other investigators. As a preliminary stage to nuclear division the chromatin is given up by the vesicles and becomes diffuse, gradually forming a loose network called a "net spireme." Chromatin granules are located on a linin network and multiply by transverse divisions. Nuclear division may then follow either one or two general types, both of which may occur in the same species, the first being amitotic, and the second similar to the primitive mitosis described by KOHL, in which a single coiled spireme segments transversely without any longitudinal splitting. The number of spireme segments is not constant.

The cortex consists of a thin colorless outer membrane investing a thick inner layer in which the pigment is dissolved, and "rightfully termed the chromatophore." The chromatophore contains cyanophycin granules and slime globules (which may be large cyanophycin granules). These are regarded as food products. The cell does not contain vacuoles. The nucleus sends out radiations which penetrate the cortex, and in certain cases also the cell wall, forming cilia.

GARDNER's investigation (4) was based almost entirely on unsectioned material. He studied a great many species, but his figures are mostly of *Oscillatoria*, *Cylindrospermum*, and *Symploca*. He considers that a nucleus is present, which occurs as three types. The most common and most primitive is the "diffuse type," con-

sisting of chromatin masses uniformly distributed throughout a ground mass. The nucleus may or may not be rather sharply delimited from the cytoplasm. The "net karyosome type," seen only in *Dermocarpa*, consists of a definite network with chromatin masses at the nodes. The nucleus of *Synechocystis aquatilis* belongs to the "primitive mitosis type," and in the resting condition is similar to the second type. In all species studied nuclear division is amitotic except in *Synechocystis aquatilis*. Here a mitosis is seen similar to that described by KOHL. Three spireme segments are formed, which become arranged parallel with the long axis of the cell. These divide transversely without a longitudinal splitting.

GARDNER describes two kinds of granules which are differentiated on the basis of their staining reactions. In the nuclei of all species examined α granules occur, except in resting cells (these may be the slime globules of other investigators). The cytoplasm of vegetative cells may or may not contain β granules, although they are invariably present in resting cells. They are regarded as reserve food (and are probably the same as cyanophycin granules). The coloring matters are held in the cytoplasm, there being no special chromatophore. The cytoplasm is fibrillar in some cases and alveolar in others.

GUILLIERMOND (5) studied *Phormidium*, *Microcoleus*, *Lyngbya*, *Scytonema*, *Rivularia*, *Calothrix*, and *Nostoc*. His observations were based almost entirely on sectioned material. The central body is regarded as a kind of nucleus (not a true nucleus), consisting of a hyaloplasm and a dense reticulum composed of an achromatic ground substance containing granules of chromatin (*corpuscules métachromatiques*). This differentiation between chromatin and achromatin was seen only in material fixed in Flemming's solution. The central body also contains one or two large nucleolus-like bodies called *corps nucleoliformes* (which probably correspond to the slime globules of other investigators). Nuclear division is amitotic, but in some respects it resembles mitotis. There is no formation of a spireme or of chromosomes, and the chromatin granules do not divide. In some of the higher species studied the chromatin granules form lines more or less parallel with each other, but the reticulum is not broken.

The cytoplasm contains granules corresponding to the cyanophycin granules of other investigators. These are of two kinds, very small ones found only on the cross walls, and large ones scattered throughout the entire peripheral region. There are no minute chromatophores. The cortex probably contains the pigments in solution, but is not organized as a single large chromatophore. In young cells the cytoplasm is homogeneous. Later small vacuoles appear, and in old cells the vacuoles fuse to form larger ones.

The investigation of Miss ACTON (1) was based on *Chroococcus*, *Gloeocapsa*, *Aphanothece*, and *Merismopedia*. Sectioned material was used. As in GARDNER'S paper, a series of transitional forms is described. The most primitive cell structure is seen in *Chroococcus turgidus*. In this species there is no definitely defined nucleus, the protoplast consisting of a ground substance and a very regular fibrillar reticulum extending throughout the entire cell. Cyanophycin granules ("plasmatic microsomes") are found at the nodes of the peripheral portion of the reticulum. Metachromatin granules occur mostly in the center of the cell. They are similar to chromatin granules in staining reactions. The metachromatin granules are connected by delicate threads which are not a part of a mitotic figure, but portions of the broken reticulum which extends throughout the entire cell. They show a tendency to accumulate as masses in the meshes of the reticulum, forming large irregular hollow granules which may correspond to the *Zentralkörner* of KOHL.

The structure of *Gloeocapsa* is similar to that of *Chroococcus turgidus*, but here the central reticulum stains more deeply than the peripheral reticulum, and occasionally stimulates a segmented spireme described by OLIVE. *Merismopedia elegans* shows an "incipient nucleus" consisting of a "restriction of deeply staining plasmatic microsomes to a small definite area in the network." This central body divides amitotically as the cell wall grows inward. In *Chroococcus macrococcus* a definite nucleus is differentiated, consisting of a reticulum with chromatin granules at its nodes. The cytoplasm contains cell sap vacuoles which break up the ground substance, the vacuolization being more marked in old cells. This vacuolization causes a breaking of the cytoplasmic reticulum and

a disappearance of the nodal points. In this species metachromatin granules are scattered throughout the cytoplasm. The nucleus divides by amitosis. The writer presents a theory of the evolution of the nucleus, which in some respects resembles that of FISCHER, quoted as follows:

> It is suggested that evolution of nucleus and cytoplasm has taken place along the following lines: The excess of food-material elaborated by the pigment was first stored in the plasmatic microsomes as a carbohydrate-cyanophycin. As more and more material was elaborated, the reserve in the central region became more complex, and the proteid metachromatin granules were formed. In time, the accumulation of nucleo-protein became restricted to a very limited area in the cell, so as to insure its equal distribution on division, and this restriction only occurred on division, as in *Merismopedia elegans*. In this way, part of the cell became physiologically and morphologically separated on account of its function in connexion with division. This area may be called the "nucleus." At a later stage the "nucleus" became stable and was always present, as in *Chroococcus macrococcus*. The ground substance also altered in character, forming a definite cytoplasm as described above.

Investigation

CENTRAL SUBSTANCE

STRUCTURE.—There is a general agreement among investigators that the living Cyanophycean cell consists of a colored peripheral portion ("cortex") and a colorless central portion ("central body"). This differentiation is very apparent in all large-celled filamentous species, and has clearly been seen by the writer also in *Gloeocapsa*. This means that the pigments are confined to the outer portion of the cell. Based chiefly upon this fact, and upon different staining reactions exhibited by the two portions of the protoplast, most investigators regard the "central body" as an independent organ of the cell, and many, particularly a number of recent investigators, consider it to be a nucleus.

The fundamental substance, or "ground mass" of the "central body," is generally considered to be distinct from the surrounding protoplasm, although a membrane separating the two portions of the protoplast is not present. Practically all of the investigators holding this view have studied only unsectioned material. As seen in sectioned cells, the "ground mass" of the "central body" is

identical in structure and in staining capacity with the substance composing the rest of the protoplast. The same substance is uniformly distributed throughout the entire cell within the cell wall. This homogeneous substance is considered to be merely undifferentiated protoplasm. Thus cytoplasm as such is not differentiated, and no definitely organized, delimited "central body" is present, as the mere absence of pigment does not give to it a structural individuality.

In addition to the undifferentiated protoplasm, the central portion of the cell invariably contains a granular substance which stains with safranin and very deeply with iron-alum haematoxylin. This substance is confined exclusively to the colorless central portion of the cell, and in *Anabaena* it often appears to reach from cross wall to cross wall. Its specificity is manifested by its constant occurrence and by its definite staining reactions. The central substance forms stringlike masses and exhibits a tendency to become locally aggregated to form granules. No resemblance to a nuclear reticulum is revealed, however, and there is no differentiation into chromatic and achromatic portions, as described by OLIVE and others. The granules, representing the denser portion of the central substance, are consequently more tenacious of the stain, but are not differentiated in any other way.

In *Gloeocapsa* the central substance presents a more reticulate appearance than in *Anabaena*, and seems to be composed of a thin fibrous portion bearing large granules at its nodal points. A careful study, however, demonstrates that there is no real reticulum in the center of the cell, but there exists merely relatively greater local accumulations of central substance to form the granules than is seen in *Anabaena*. OLIVE states that in the "spireme" of *Oscillatoria* "the achromatic and chromatic substances seem to be blended," but "it is probable that it is made up of a definite number of distinct chromatin granules arranged along a linin thread."

Because various portions of the stringlike masses of central substance are not sharply differentiated as conspicuous granules in *Anabaena* and other genera, the masses often present the appearance of chromosomes. A method which would cause the central substance to be stained too intensely might tend to exaggerate this

illusion, and might give one a basis for thinking that chromosomes are organized in the Cyanophycean cell. Very frequently also, and for the same reason, the appearance of a spireme is simulated in the organization of the central substance (fig. 3). In *Gloeocapsa* this illusion is commonly presented (fig. 12). Possibly instances of this kind correspond to the spireme formation which OLIVE sees in "favorable cases," and which he states is better shown by *Gloeocapsa* than by any other form with which he worked. Miss ACTON is in close agreement with this interpretation:

> The rodlike chromosomes of KOHL can hardly be explained as central granules, though stained preparations, in which under a $\frac{1}{12}''$ objective only central granules can be seen, do sometimes under a $\frac{1}{6}''$ objective show structures simulating karyokinetic figures with rodlike chromosomes; with the greater magnification the rodlike bodies are resolved into granules.

In the species of *Anabaena* studied no large granules were seen in the center of the cell corresponding to the large *Zentralkörner* of KOHL. Because this investigator failed to observe them in *Nostoc*, and in *Anabaena* identified the many small central granules with the *Zentralkörner* of *Tolypothrix*, it is probable that these are merely the granules of the central substance. As already pointed out, ZACHARIAS (13) is in agreement with this interpretation. Structures corresponding to *Zentralkörner* (or slime globules), however, have been seen in the central portion of the cell by other investigators, and hence they may be constant features of the cell structure of other genera.

DIVISION.—Most of the investigators agree that cell division in the Cyanophyceae is accompanied by the formation of a cell wall which develops centripetally as a ring. The relation between the formation of the cross wall and the division of the "central body," however, and also the mechanism of its division are matters of wide difference of opinion.

In *Anabaena* the division is begun by a slight elongation of the cell. A ringlike ingrowth then appears, which is the beginning of a cross wall, but no correlative change is seen in the behavior of the central substance (figs. 2*b*, 4). The cell wall then develops farther and the central substance becomes constricted into two approximately equal masses (fig. 5). It should be noted that the growth

of the cross wall does not mechanically constrict the central substance, but merely accompanies its division. In *Gloeocapsa* a series of similar stages is seen (figs. 13–16), but here the division of the central substance occurs relatively earlier with reference to the development of the cross wall. OLIVE states that cell division in *Gloeocapsa* is effected by simple constriction and not by a centripetally growing wall. The present study clearly demonstrates that such is not the case.

The process of cell division is strictly amitotic, there being no formation of chromosomes, spindle fibers, or of anything suggestive of a mitotic mechanism. An approximately equal distribution of the central substance to each daughter cell is effected without the precision which characterizes the separation of chromatin in the higher plants. This interpretation is in harmony with the results and conclusions of ZACHARIAS, FISCHER, GARDNER, GUILLIERMOND, and Miss ACTON. FISCHER, GUILLIERMOND, and Miss ACTON further agree that the process of cell division often simulates a mitosis.

HEGLER, KOHL, and PHILLIPS describe chromosomes which correspond to the stringlike masses of central substance. This resemblance has already been discussed. OLIVE, however, considers each granule of the central substance to represent a chromosome, the number of which is constant. These divide by longitudinal fission with each "mitosis," this behavior being best seen in *Gloeocapsa*. The present investigation furnishes no evidence that the granules of the central substance are autonomous bodies. This matter has been a special subject of study, particularly in *Gloeocapsa*, and it is positively certain that no behavior occurs corresponding to that described by OLIVE. Obviously there is not the definiteness about it which characterizes mitosis in the higher plants. In fact, many of the conclusions of OLIVE regarding cell division are stated provisionally and with considerable uncertainty.

NATURE.—The central substance has been most generally regarded as chromatin chiefly on the basis of its reactions to stains and to microchemical tests, and its behavior in cell division, although there is considerable difference of opinion regarding its nature and organization. An extreme view is that of FISCHER, who considers the central substance to be a carbohydrate which he calls "ana-

baenin," a transformation product of glycogen. This reacts quite unlike the chromatin composing the chromosomes of *Lilium* to various nuclear stains, iodine solutions, and other reagents. All of the other chief investigators regard the central substance as protein, and consider it to be either chromatin or metachromatin, a substance similar to it.

The only facts disclosed by the present study which might be significant in a determination of the nature of the central substance are its stronger affinity for certain cytological stains than the protoplasmic ground substance, and its behavior in cell division. No comprehensive or comparative study with a higher plant in regard to staining reactions was undertaken, as FISCHER has done, and no microchemical tests were made. Thus it is not possible to state definitely whether or not the central substance is identical or closely similar to the chromatin of the nuclei of the higher plant cells, although its behavior in cell division suggests that it may have functions analogous with chromatin. It is called chromatin by most investigators, however, on no better grounds than those mentioned.

Whether the central portion of the cell constitutes a nucleus depends upon whether it really has morphological individuality, as has been claimed. The lack of delimitation between the central and peripheral portions of the cell has already been discussed, and the conclusion is reached that there is no evidence that a "central body" exists as an independent organ of the cell. This fact has been established through an exhaustive study of sectioned material. If one be willing to apply the term nucleus to a mass of granular material, scattered throughout the central portion of the cell without a definite organization, and with no delimitation in ground substance from the peripheral portion, a nucleus may be said to exist in the Cyanophyceae. It is merely a matter of terminology. It seems more reasonable, however, to consider that a nucleus is represented in the Cyanophycean cell by a substance which resembles chromatin in several ways, but without the organization of a nucleus. This substance may have some or all of the functions of chromatin, and thus its division may anticipate the exact mitotic mechanism of a definite nucleus.

Ground substance

Structure.—The fundamental substance, or undifferentiated protoplasm of which the greater part of the Cyanophycean cell is constructed, presents a distinctly granular appearance in sectioned material, the granules being exceedingly minute. Stained preparations of cells mounted whole show considerable diversity in the relative staining capacity of the ground substance of cells of different colonies, some cells being deeply stained and others very lightly, and the impression is obtained that the ground substance varies in density. In sectioned material, however, no such disrepancy is seen, the protoplasts being uniform in their affinity for the stain. This is probably due, not to the sectioning itself, but to the use of more careful methods in making the preparations.

The ground substance, like the protoplasm of most plant cells, is highly vacuolated. The vacuoles in *Gloeocapsa* are very small as compared with the size of the cells, and normally there are many of them, not merely a few large ones. In *Anabaena* some of the vacuoles are much larger than the others. Vacuolization of the protoplasm can be satisfactorily demonstrated only by the use of sections. Vacuoles appear very indistinctly in unsectioned cells. This fact may account for the failure of most of the investigators using unsectioned material to find any vacuoles except large ones in old enfeebled cells.

The protoplasm of the cells of the Cyanophyceae is not reticulate but alveolar. Miss Acton has described a fibrillar reticulum extending throughout the peripheral region of the cell in some of the Chroococcaceae and throughout the entire cell in others, but the writer has seen nothing in the Cyanophycean cell resembling a reticulum. Threadlike masses of protoplasm are seen between the vacuoles, but these are obviously granular and not fibrillar. It is highly probable that the radiations extending from the "central body" to the cell wall, which are described and figured by Fischer, Kohl, Phillips, and others, are merely threadlike masses of undifferentiated protoplasm occupying the spaces between the larger vacuoles (fig. 3).

Pigments.—The organization of the coloring matters in the cell has given rise to as much controversy as the structure and

division of the "central body." HEGLER and KOHL see a large number of extremely minute peripheral chromatophores, GARDNER and GUILLIERMOND think that the pigments are diffused in the "cytoplasm," while FISCHER, OLIVE, and PHILLIPS regard the entire protoplast exclusive of the "central body" as a single, large, definitely organized chromatophore. In reviewing the various opinions concerning the occurrence of the pigments, it is important to realize that minute chromatophores have been seen mostly by those using unsectioned material.

In the present investigation no minute chromatophores have been seen in the peripheral portion of sectioned cells, or any minute bodies which might be interpreted as representing chromatophores. In living cells the presence of definitely organized colored plastids imbedded in colorless protoplasm cannot be demonstrated under the highest magnification. The peripheral region presents a uniformly blue-green appearance. For the reasons mentioned, it is thought that the blue and green pigments are held in solution in the peripheral ground substance. It is very probable that the coloring matters are dissolved in the cell sap of the vacuoles. KOHL notes that the minute chromatophores seen by him are ordinarily in contact with each other, thus giving the protoplasm an alveolar appearance. Inasmuch as he fails to see vacuoles except in rare cases, it seems likely that his minute chromatophores are vacuoles containing pigments dissolved in the cell sap.

Whether or not the peripheral portion of the cell constitutes a true chromatophore is merely a matter of interpretation. In the cells of the higher green plants chromatophores occur as definitely organized, pigment bearing, protoplasmic bodies, and represent one or more differentiated portions of the protoplast where the process of photosynthesis has become localized. It is impossible to apply this conception to an interpretation of the structure of the colored portion of the Cyanophycean cell. In these primitive plants photosynthesis is a general function of the peripheral protoplasm. This region cannot be regarded as an independent organ of the cell because it has no definite form, is not sharply delimited from the central region, is not surrounded externally by a layer of colorless protoplasm, and is differentiated from the central region only by

the possession of pigments. If the peripheral region be thought to represent a single large chromatophore, one must also, with FISCHER, regard the central region as cytoplasm, or one must hold the view that the "central body" is a nucleus and the cell has no cytoplasm. OLIVE and PHILLIPS, however, inconsistently call the peripheral region cytoplasm organized as a chromatophore, yet do not state their conception of the nature of a chromatophore. It would seem that the simpler interpretation just suggested would make this less a matter of terminology and more in harmony with observed facts.

CYANOPHYCIN GRANULES.—In addition to the central substance and the protoplasmic ground substance, the Cyanophycean cell usually contains a number of bodies most generally called cyanophycin granules. These are confined to the peripheral region, and are never found in the center of the cell. FISCHER sees them in some cases in the central region, but what he describes as cyanophycin granules in the center of the cell are probably the *Zentralkörner* of KOHL, or the slime globules of other investigators. The limitation of the cyanophycin granules to the peripheral region can be demonstrated in thin median cross-sections (figs. 3, 6, 7).

The cyanophycin granules are metaplasmic inclusions representing reserve food, and are thus regarded by most investigators. They are generally considered albuminous, but ZACHARIAS and Miss ACTON think that they are carbohydrate in nature. HEGLER, KOHL, and FISCHER describe the cyanophycin granules as albuminous crystalloids. These and other investigators have demonstrated that the first visible product of photosynthesis is glycogen, and that the cyanophycin granules represent reserve food. Their accumulation in resting cells is a matter of common observation (fig. 6).

Cyanophycin granules are entirely absent from some cells, in others they are very abundant, and all intermediate conditions are seen. Moreover, this variation may be seen in the cells of the same filament. When the cell contains just a few of them, they are very small and stain deeply. As they increase in size, they lose their capacity to stain intensely with iron-alum haematoxylin, and when large stain only around the outside. This probably results from the fact that they seem to be hollow when large. With this

stain, however, the cyanophycin granules are never colored as deeply as the central substance. When safranin is followed by gentian violet, the central substance stains red and the cyanophycin granules purple. In *Gloeocapsa* no large cyanophycin granules were seen in the material studied, but nearly all of the cells contain smaller peripheral granules which probably represent reserve food.

A relationship between the cyanophycin granules and the central substance has not been demonstrated. FISCHER and Miss ACTON have presented theories dealing with a possible relationship, and these are suggestive of a mode of origin of the nucleus of the higher plants. According to these theories the central substance represents surplus food elaborated in the peripheral region, and which was first stored there as a carbohydrate. This then moved into the central region and became more complex. Later it was transformed into a protein substance which became equally divided between the daughter cells. Finally, as a nucleus was organized the central substance came to have new functions.

Summary

1. The cell of the Cyanophyceae possesses no differentiated central organ of the nature of a "central body" or a nucleus. Undifferentiated protoplasm is uniformly distributed throughout the entire cell, the peripheral portion differing from the central portion merely in the presence of pigment.

2. A deeply staining granular substance is present in the central region, which forms stringlike masses somewhat resembling chromosomes. The central substance is homogeneous and shows no differentiation into chromatic and achromatic portions, and no resemblance to a nuclear reticulum.

3. Cell division is accomplished by a centripetally growing cell wall. As this develops, the central substance becomes constricted into two approximately equal masses. The division of the central substance is strictly amitotic. The central granules have no individuality.

4. The central substance resembles chromatin in several ways and it may have similar functions, but it is not organized as a nucleus.

5. The ground substance, or undifferentiated protoplasm, is minutely granular and highly vacuolated. It does not exhibit a reticulate structure.

6. The pigments are held in solution in the peripheral protoplasm, probably in the cell sap of the vacuoles. There is no organized peripheral chromatophore.

7. The peripheral protoplasm contains cyanophycin granules representing reserve food. These may be absent or present in varying degrees of abundance, even among cells of the same filament. They accumulate in resting cells.

8. The Cyanophycean cell is organized without the differentiation seen in the cells of the higher plants. A slight functional differentiation is displayed in the restriction of photosynthetic activity to the peripheral region of the cell, and in the presence of a substance in the center of the cell which is transmitted through successive generations.

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EXPLANATION OF PLATE VII

FIG. 1.—*Anabaena circinalis:* sketch of filament mounted whole, showing its general form, sheath not indicated; ×440.

FIG. 2.—*Anabaena circinalis:* median longitudinal section of three cells, showing central substance (chromatin?) and peripheral cyanophycin granules, protoplasmic ground substance highly vacuolated; *a*, *c*, cells not in division; *b*, cell showing early stage in division; ×3000.

FIG. 3.—*Anabaena circinalis:* median cross-section, showing same features; ×3000.

FIGS. 4, 5.—*Anabaena circinalis:* median longitudinal sections, showing later stages in cell division; ×3000.

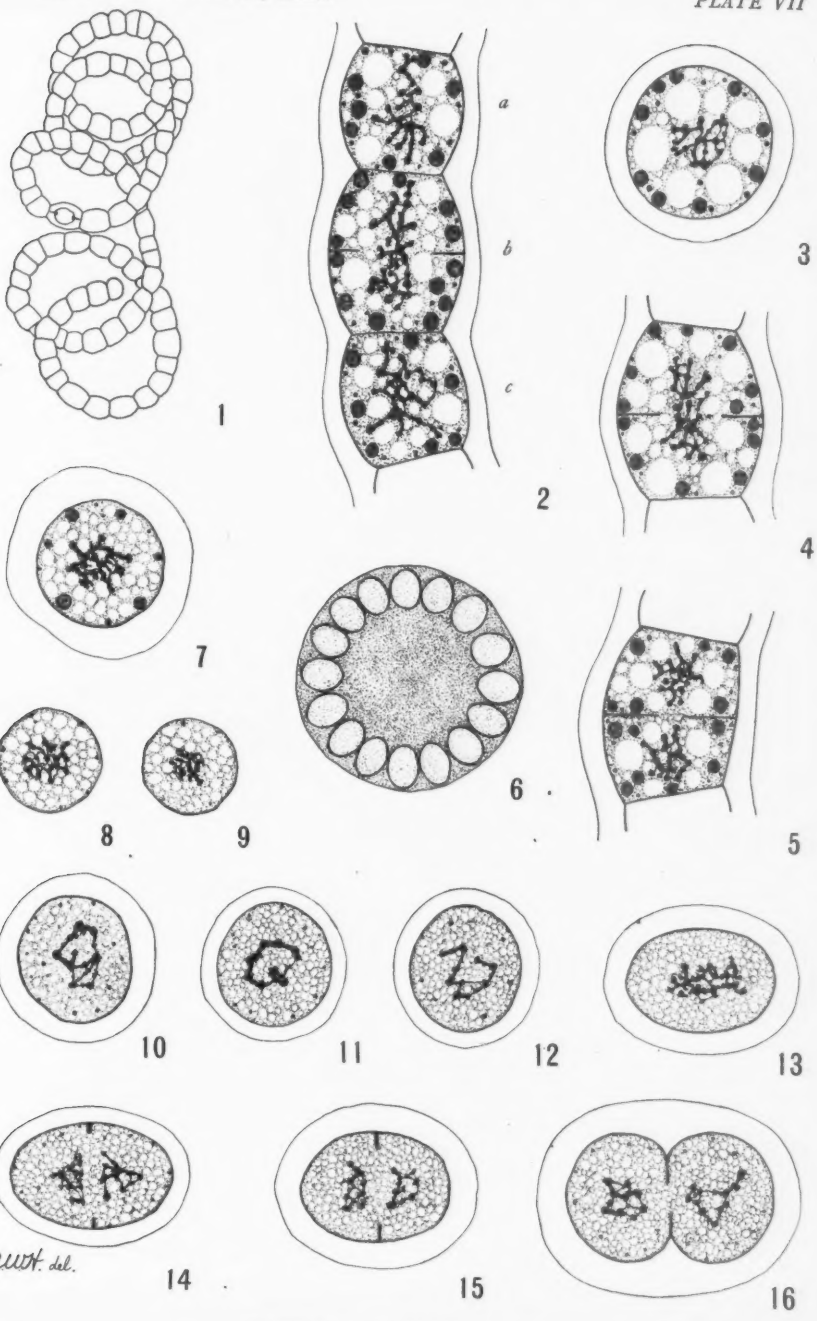
FIG. 6.—*Anabaena circinalis:* cross-section of resting cell ("spore"), showing extremely large peripheral cyanophycin granules; ×3000.

FIG. 7.—*Rivularia pisum:* cross-section of cell near base of filament, showing central substance and large cyanophycin granules; ×3000.

FIGS. 8, 9.—*Rivularia pisum:* cross-sections of cells a short distance away from base of filament, showing central substance and no large cyanophycin granules; ×3000.

FIGS. 10–12.—*Gloeocapsa aeruginosa:* sections of cells not in division; ×3000.

FIGS. 13–16.—*Gloeocapsa aeruginosa:* sections of cells in successive stages of cell division; ×3000.



HAUPT on CYANOPHYCEAE

SPERMS OF VALLISNERIA SPIRALIS

ROBERT B. WYLIE

(WITH PLATES VIII–X)

Information regarding fertilization in higher plants is of fundamental importance. One of the first considerations in this connection is the morphology of the gametes themselves. The egg has offered little difficulty, as its major parts are obvious, but the sperm of the angiosperm does not reveal itself with equal clearness. Its cramped quarters in the pollen tube and the frequent inconspicuousness of the male gamete, except in fertilization stages, have focused attention primarily upon the sperm nucleus rather than upon the gamete as a whole. Of the hundreds of papers dealing with the gametophytes of angiosperms, very few give details regarding the organization of the male gamete previous to conjugation of the sex nuclei.

In his classic paper, NAWASCHIN (2) figured sperms as cells in the pollen tubes within the ovules of *Juglans regia* and *J. nigra* displaying zones of lighter staining cytoplasm immediately surrounding the male nuclei. In the higher angiosperms, however, he found no trace of such cytoplasm associated with the sperm nuclei after their entrance into the ovule.

BLACKMAN and WELSFORD (1), in their study of fertilization in *Lilium Martagon*, agree with the findings of NAWASCHIN for that species, and report only nuclei representing the sperms in the terminations of the pollen tubes. Subsequently WELSFORD (3) made a detailed study of spermatogenesis in *Lilium auratum* and *L. Martagon*, tracing the formation of the male cells, reaffirming the disappearance of the cytoplasm from about the sperm nuclei, and supporting the view that the sperms enter the embryo sac as naked nuclei.

A number of years ago the writer (4) found conclusive evidence that the sperms of *Elodea canadensis* remain intact, as complete cells, during their journey through the pollen tube in the ovary;

but their history after the pollen tube had entered the ovule could not be traced with any satisfaction from that material, as the ovules are few in number and flowers must be sectioned singly. Subsequently, *Vallisneria spiralis*, which has very large numbers of ovules, was tried, and its sperms were found equally favorable for study, but there were difficulties in securing properly fixed material. While any favorable preparation showed male cells in the pollen tubes, events taking place within the ovule were uniformly obscured. More recently these difficulties have partly been overcome through better fixation, and the history of the male cells in this species may now be outlined.

The orthotropous ovules of *Vallisneria* are borne numerously in the long-stalked epigynous ovary (5). They are attached laterally to the walls, and the ovarian chamber surrounding them is filled with a gelatinous material which is highly resistant to many reagents, but dissolves slowly in water. If entire ovaries are killed, even with walls shaved thin or partly removed, the matrix surrounding the ovules retards fixation for a long time. Cutting the ovary into short pieces before fixation affords no better results. This slime dissolves so slowly that fertilization is completed and the triple fusion far advanced before the killing solution reaches the nuclei of the embryo sac. Ovaries killed *in toto* in Flemming's stronger solution, however, yielded fine preparations of the pollen tubes lying near or against the ovary wall. In this situation, covered by little gelatin, they were reached readily by the reagents, although the ovules lying deeper in the same ovary would be very poorly fixed.

Experiments later showed that if the ovary were cut across near the middle, it was possible to squeeze out the ovules imbedded in this gelatinous matrix. The gelatin might then be dissolved in water or in aqueous chromacetic mixtures, thus setting the ovules free. Penetration of the killing agent was delayed, however, and preparations from such material invariably gave only the later steps of fertilization, although post-fertilization stages were well fixed. Obviously all ovules that had received pollen tubes before being squeezed out of the ovary had time to complete the conjugation of their gametes before fixation took place.

Recently suitable material for the study of fertilization was secured by chilling the masses of ovules while dissolving out the gelatin and during fixation. In this way it was possible to retard growth and cyclosis, and so to secure all stages in the fertilization process. Freshly pollinated flowers were selected, and the expressed contents of the ovaries were put into a large beaker of ice water. Since the ovules of scores or hundreds of flowers were used, it was necessary to stir the water for several hours, decanting from time to time, and adding fresh quantities of cold water as the gelatin dissolved. At best, however, much slime remained after several hours' washing. Ovules expressed at 7:00 P.M. were killed five hours later by pouring strong Flemming solution over them, after straining off all the slimy mixture that could be gotten through bolting cloth suitably meshed to hold back the ovules. Following fixation the ovules were washed, dehydrated, and imbedded *en masse* in paraffin, and blocks from the mass were sectioned parallel to the lower surface of the cake. By using paraffin of intermediate melting point, sections 15–30 μ thick were prepared which with light triple stain gave favorable views of the embryo sac and contents. Thinner sections were used for the study of critical stages. This method has been outlined in the hope that it may be found helpful in securing fixation of resistant material where growth is carried beyond the desired stage owing to delayed penetration. Refrigeration retards development while the killing agent is penetrating to the interior tissues.

The method of pollination insures to the stigmas a liberal amount of pollen, provided staminate flowers are at hand. The microspores are sticky and adhere as a pollinium, which is delivered to the stigmas by the floating staminate flower. Field observation shows that in areas favorable for the growth of *Vallisneria* there are commonly many inverted pollen bearing flowers inclosed with each pistillate flower when it is submerged, following the period of exposure at the surface of the water (5). This results in crowds of pollen tubes, which literally race for the ovary and enter its cavity in great numbers. Sections may show many tubes in the upper portion of an ovary, while the lower end is still entirely devoid of these structures.

The main course of the pollen tubes is along the interior of the ovary wall in close proximity to the glandular cells lining its surface. In this peripheral region they show as nearly straight lines, often so numerous as to form a compact layer, especially in the stylar region. This favors their fixation, as already noted, while the deeper lying ovules buried in the gelatin are not promptly reached by killing reagents. Tubes diverge into the ovarian chamber at all levels, and these are much more numerous than the ovules, although their relative abundance could not be determined with certainty, as doubtless many of them are lost from the sections. The tubes within the cavity are much more crooked than those running along its walls. Tubes entering ovules persist adjacent to the micropyle for some time after fertilization, suggesting that they may live parasitically upon the contents of the ovule, a situation previously reported for *Elodea canadensis* (4). While most of the pollen tubes pass directly into the ovules, some are very crooked and contorted near the micropyle.

The sperms of *Vallisneria spiralis* are formed by division of the generative cell while yet within the pollen grain (fig. 11). During the time they are in the microspore they are considerably elongated, and are obviously connected by their adjacent tips. This attachment holds until the sperms enter the embryo sac, and insures the simultaneous entrance of the two sperms for the work of fertilization. The male cells emerge from the pollen grain in somewhat contracted form, and during their progress through the short style they remain almost isodiametric (fig. 1). As the tube enters the ovarian chamber they begin to elongate again and soon assume rather striking proportions (figs. 4, 6). In spite of their extreme elongation the sperms remain attached together while in the pollen tube. The relative position of tube nucleus and paired sperms in the pollen tube seems to be a matter of chance. Based on a considerable number of observations it was noted that the tube nucleus follows the sperms about as frequently as it precedes them. In all cases, however, the vegetative nucleus remains near the male cells, and in one instance it was found alongside of the sperms in a somewhat enlarged tube in the style (fig. 2).

In a study of *Elodea canadensis* the writer (4) identified the sperms as male cells in supernumerary pollen tubes forming peculiar tuber-like enlargements, termed "cystoids," within the ovarian chamber. Corresponding structures have also been found in the ovaries of *Vallisneria spiralis*, and the presence of male cells, still intact, in these cystoids (figs. 9, 10) strengthened the belief that with proper fixation the presence of cytoplasm about the male nuclei inside the micropyle might be demonstrated. Sections of thousands of ovules, killed in various ways, were examined in vain, however, as in all cases the fertilization process was far advanced before the killing agent reached the interior of the ovule. It was only through the use of material treated with ice water during fixation, as previously described, that events transpiring within the ovule could be determined.

Definite male cells of *Vallisneria* inside the micropyle were first noted emerging from the tips of pollen tubes that had entered the ovule after fertilization had been achieved. The second tube enters the remaining synergid, and its contents seem to be discharged more slowly, for repeated instances were observed in which the pair of sperms could plainly be seen occupying the tip of the tube, or emerging from its protoplasm. In all cases the sperms showed clearly as complete cells (fig. 12). The relatively lighter zone of cytoplasm surrounding the male nucleus was sharply delimited externally by the darkly staining contents of the pollen tube. The form and size of the male cells in these tubes were approximately that shown in the germinating microspore, namely, oval and shortened rather than attenuated as they are in the intervening portion of the tube. These supernumerary sperms remain at the tip of the pollen tube, and in no instance were they found in other parts of the embryo sac, so that there is no evidence of polyspermy.

In several preparations the male cells were likewise identified emerging from tips of pollen tubes first to enter the embryo sac. The initial tube to enter an ovule terminates in one of the synergids, and the emerging sperms lie side by side or very close together (fig. 13). Although they escape simultaneously, no connection

could be seen after leaving the tube, and their separation soon takes place. The end of the pollen tube seems to break open, and portions of its contents escape with the gametes. Irregular patches of darkly staining material lie near by, and at this stage the fixed material shows a more or less indefinite boundary at the lower end of the tube, in sharp contrast with earlier stages when it presents a very definite and rounded outline. The relative infrequency of slides showing sperms emerging from the initial tubes to enter ovules suggests that this period is passed quickly, and that the male gametes are soon advanced to the fertilization stage.

Following their emergence from the pollen tube as complete cells, the sperms are soon separated. One flattens against the egg membrane, while the other is carried down along the angle formed by the remaining synergid and egg, and thence out into the embryo sac. The male cell destined to unite with the egg was favorably seen in many instances, either near the tip of the tube or in contact with the egg (figs. 12, 13). In these situations, the male gamete showed very definitely its cytoplasm, surrounding the nucleus, even when flattened against the egg. Both egg and sperm at this stage showed clearly their morphology as complete cells, with only a membrane between the two protoplasts.

No section was found showing events as the limiting membrane separating the male and female cells broke or yielded. Their union would doubtless be accomplished quickly, and perhaps only the study of living material could show the actual history of events in their conjugation. It seems certain, however, that some or all of the sperm cytoplasm would enter the egg with the male nucleus. Following the union of the two cells their nuclei unite. The male nucleus approaches the female nucleus in rounded form (fig. 8), and their fusion is far advanced by the time the second sperm reaches the polars. While there may be suggestive differences in the appearances of the vacuoles within the cytoplasm of the zygote, one could not identify male and female portions of this mass. The second sperm seems to lose its cytoplasm about the time it leaves the egg apparatus. Numerous preparations showed this sperm nucleus in successive positions as it approached the polars. All the male nuclei seen lying in the strand of cytoplasm leading to the

polars appeared to be naked, and were nearly spherical in form. They were always found imbedded in this strand of cytoplasm joining egg apparatus with polars, and are doubtless carried along by the streaming of the protoplasm. This journey seems to take some time, for the triple fusion takes place considerably later than the union of egg and sperm. Fixation methods that show no fertilization stages may show all the later phases of "double fertilization" in the same material. The nuclei of the male cells are always rounded, although somewhat elongated during the journey through the pollen tube. Following emergence they are oval, the second sperm nucleus soon becoming spherical. There is no suggestion of motility at any stage, thus offering sharp contrast with the vermiform nuclei so often figured.

The tube nucleus should be discussed, since its progress with the sperms was followed in this study. Spherical while in the pollen grain (fig. 11), it elongates very much as it passes down the pollen tube into the ovary (figs. 3, 5). The degree of elongation is most striking, the nucleus showing a length as great as 147 μ while only 5.5 μ in diameter (fig. 14), or nearly 27 times as long as wide.

The writer believes that in *Vallisneria spiralis* the remains of the tube nucleus constitute the *x*-bodies so conspicuous in the tips of pollen tubes after fertilization. The emergence of the sperms as male cells from the pollen tubes could leave behind in the pollen tube no cytoplasm from these gametes, which is held by WELSFORD (3) to be their origin in *Lilium*. Moreover, the contents of the cystoids lying among the ovules contribute evidence as to the genesis of the *x*-bodies. Cystoids formed on pollen tubes in which the sperms precede the tube nucleus may contain male cells, but show no *x*-bodies if the vegetative nucleus is still out in the tube. One instance was found in which the tube nucleus was partly in the cystoid, and it seemed to have disintegrated as it advanced into the chamber containing the male cells (fig. 9). Later stages show broken portions of the tube nucleus, quite comparable to the *x*-bodies included with the sperms in the cystoid (fig. 10). These preparations also reveal a series of changes in the *x*-bodies within the synergid. Relatively inconspicuous at fertilization stage, owing to the darkly staining content of the tube, they become much more

marked in post-fertilization material where they are usually prominent.

Discussion

Vallisneria, except for the initial difficulty of proper fixation, offers favorable material for the study of gametes and fertilization. The ovules are produced in large numbers, are readily removed from the ovary, and may be imbedded in masses for sectioning. The male cells, which are organized in the pollen grain, may be traced as complete cells through the pollen tube, into the embryo sac, and in the case of the first sperm, up to the fertilization stage. The second sperm loses its cytoplasm before its conjugation with the polars.

There is a difference in the behavior of the gametes from an initial tube as compared with those released from a second tube to enter the embryo sac. From the former the sperms seem to emerge quickly, as relatively few preparations show sperms within the ovule other than in conjugation with the cells of the female gametophyte. On the other hand, secondary pollen tubes, while not of common occurrence, frequently show sperms in close association with the tip of the tube. This suggests that for some reason the male gametes from secondary pollen tubes are either not discharged or are left in the vicinity of the synergid. In this species, therefore, it seems that some condition acts automatically as a check to polyspermy.

In this connection it should be noted that NAWASCHIN (**2**) in his study of *Juglans* was working for the most part with the contents of secondary pollen tubes containing such extra sperms. His figs. 22, 23*b*, 26, 29, 31, 34, 35, and 39 show supernumerary sperms due to the entrance of a second pollen tube into an embryo sac, while his fig. 35 also shows an ovule containing a third pollen tube which had not yet reached the embryo sac.

Although there may be no valid objection to conclusions based on the morphology of these non-functioning or supernumerary sperms, greater confidence no doubt should be placed on findings related to the primary sperms such as actually function in the fertilization process. NAWASCHIN figures such primary male cells in a few instances. His fig. 25 and fig. 33 show sperms in a pre-

fertilization stage which seemingly have cytoplasm about the male nuclei, although from the figures evidence for such investing cytoplasm rests mainly on the observed clear space between the male nucleus and the adjacent contents of the tube or embryo sac, since the structural presence of cytoplasm is not represented in the drawings. In *Vallisneria* similar conditions are presented, but in this form there is conclusive evidence of the presence of a sperm cytoplasm about the male nucleus.

Although the writer has thus far been unable to demonstrate male cells in the embryo sac of *Elodea*, he has little doubt, based on the similarity of conditions in general, and especially within the cystoids, that they do not differ from those found in *Vallisneria*. The chief difficulty in working with *Elodea* is that of securing large numbers of ovules, as there are but four or five in one flower, and these cannot be removed and massed as in the case of *Vallisneria*.

This record of functioning male cells in *Vallisneria* (and their presumptive presence in *Elodea*) is not in harmony with NAWASCHIN'S suggestion that this character is associated with chalazogamy. It is, however, in agreement with his conclusion that such sperms are likely to be found in the more primitive phyla of angiosperms, suggestive of the condition found in the majority of gymnosperms. Both *Vallisneria* and *Elodea* belong in the Helobiales, one of the more primitive divisions of the monocotyledons.

This study leads to the conclusion that the *x*-bodies found within the embryo sac in *Vallisneria* may have come from the disintegration of the tube nucleus. Their number, size, and shape are variable, and conform closely to similar bodies found in the cystoids containing male cells lying alongside the ovules in the ovarian cavity. NAWASCHIN (**2**) shows (fig. 37) a tube which had not yet entered the embryo sac containing, in addition to the sperms, a number of brightly staining bodies, quite comparable to *x*-bodies which he says were formed from the disintegrated tube nucleus. He does not, however, suggest any connection between these structures in the pollen tube and the *x*-bodies within the embryo sac.

The presence in an angiosperm of sperm and egg in conjugation, with both gametes showing plainly as complete cells, is of interest from more than one point of view. In the first place it is important

to determine the series of events following rupture of the membrane separating the two gametes when fertilization actually takes place. It may be that such can be determined only through direct observation of living material, a method involving serious difficulties in this form. The observed conjugation of protoplasts in lower plants affords evidence that part or all of the cytoplasm of the sperm probably enters the egg cell of *Vallisneria* at the time of fertilization. In proportion as the male cytoplasm combines with that of the female gamete, its possible hereditary significance becomes a matter of concern to the geneticist. Related to this, but quite apart from hereditary considerations, is the possibility that the foreign cytoplasm may exercise some physiological influence upon the development and nutrition of the zygote. Meanwhile the morphologist, with no less interest in these theoretical aspects of the question, desires fuller information as to the structure of the sperm in angiosperms in general, and its value as a criterion for consideration of relationships between members of this group. Recalling the thousands of ovules examined in this study and the long series of failures before suitable material was secured, it may be that special methods, adapted to the species under study, may reveal more fully the morphology of the sperm in the higher seed plants.

Summary

1. The male cells are organized in the pollen grain following division of the generative cell, and are united by their tips until the embryo sac is reached.

2. The sperms become greatly elongated during their journey through the pollen tube.

3. The tube nucleus may either precede or follow the sperms in the pollen tube.

4. The tube nucleus becomes greatly elongated after leaving the pollen grain, and in its later disintegration probably contributes the *x*-bodies so conspicuous in the tips of pollen tubes in the embryo sac.

5. Pollen tubes within the ovarian chamber often form tuber-like enlargements at their tips containing male cells and also *x*-bodies obviously derived from the tube nucleus.

6. The sperms are released as distinct male cells from the tip of the pollen tube after it enters a synergid.

7. The sperm which unites with the egg possesses a cytoplasm about its nucleus when this gamete comes in contact with the egg cell.

8. The second sperm loses its cytoplasm shortly after it enters the embryo sac, and its naked spherical nucleus is carried to the polars in a strand of cytoplasm.

9. A second pollen tube frequently enters the ovule, and this discharges into the remaining synergid. Its male cells seem to remain near the tip of the tube and there is no evidence of polyspermy.

10. The possession of male cells as gametes is not necessarily associated with chalazogamy as reported by NAWASCHIN, but may be limited to the more primitive orders as he suggests.

11. The union of the male and female gametes as cells probably means that some or possibly all of the cytoplasm of the male cell enters the egg at the time of fertilization.

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EXPLANATION OF PLATES VIII–X

Abbreviations used in describing figures are as follows: *a*, pollen tube; *b*, male cell; *c*, tube nucleus; *d*, egg; *e*, *x*-bodies; *f*, synergid; *g*, sperm nucleus; *h*, fusion nucleus.

PLATE VIII

FIG. 1.—Portion of pollen tube in stylar region, showing male cells joined.

FIG. 2.—Portion of pollen tube in stylar region, showing tube nucleus lying beside male cells.

FIGS. 3, 5.—Portions of pollen tubes in stylar region, showing tube nuclei and male cells.

FIGS. 4, 6.—Elongated male cells within pollen tube in ovarian chamber.

PLATE IX

FIG. 8.—Micropylar end of embryo sac, showing pollen tube, one synergid, egg, and sperm nucleus in contact with nucleus of egg.

FIG. 9.—Pollen tube in ovarian chamber, with cystlike enlargement at end; male cells and portion of disintegrating tube nucleus in cyst.

FIG. 10.—Cystoid, showing male cells and disintegrated tube nucleus.

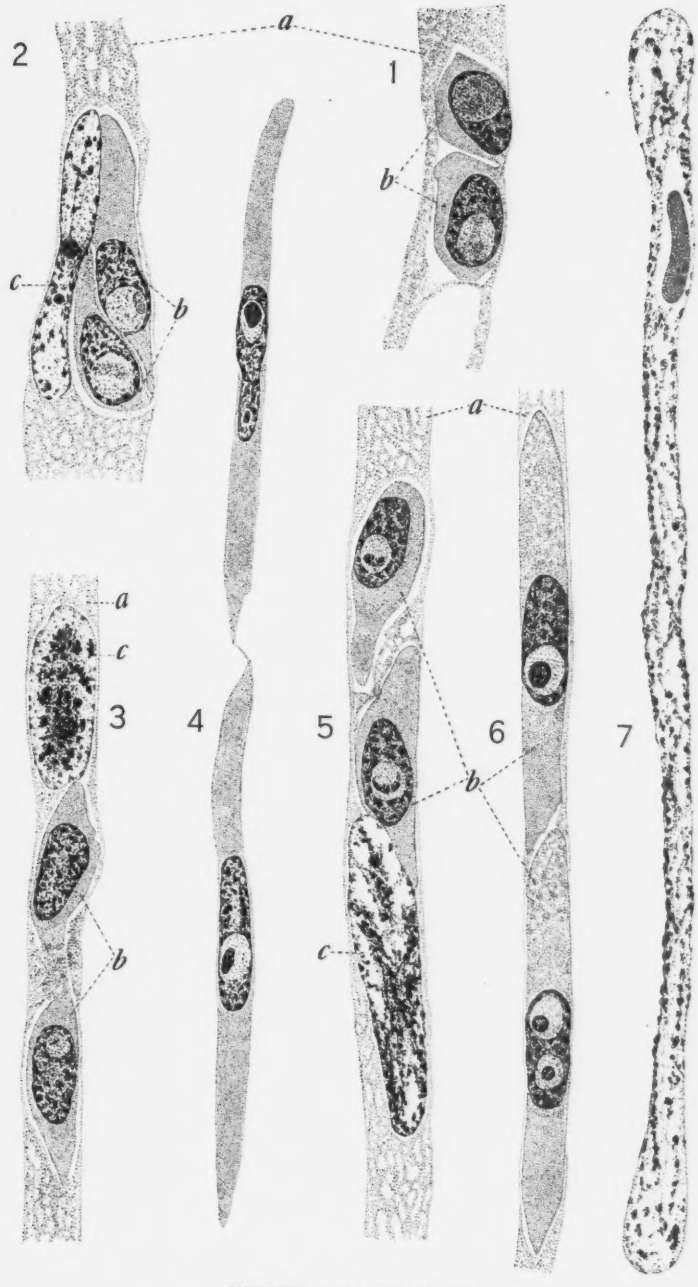
FIG. 11.—Pollen grain, containing tube nucleus and male cells.

PLATE X

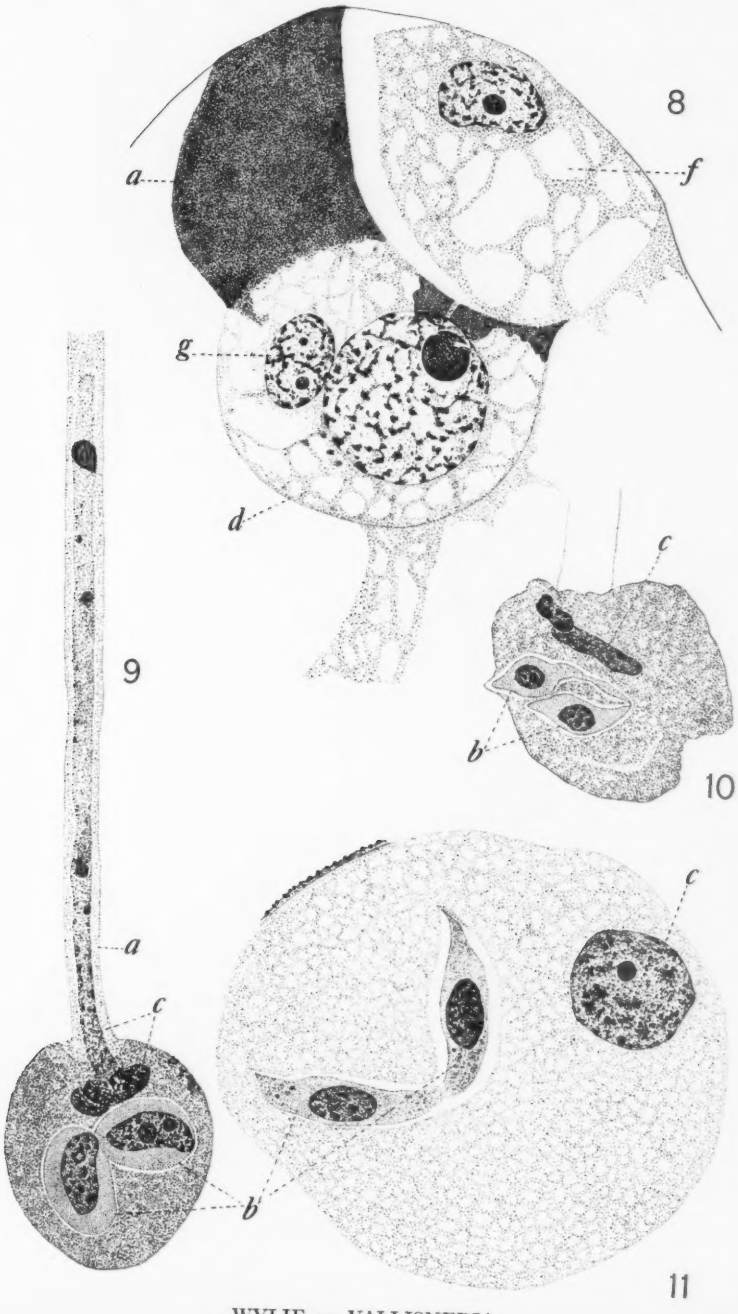
FIG. 12.—Micropylar end of embryo sac, showing two pollen tubes, one of them releasing male cells; egg and fusion nucleus in outline; sperm nuclei from first pollen tube seen in fertilization and "double fertilization" stage.

FIG. 13.—Micropylar end of embryo sac, showing one pollen tube containing $\varkappa$-bodies, and releasing two male cells, one synergid and egg cell in outline.

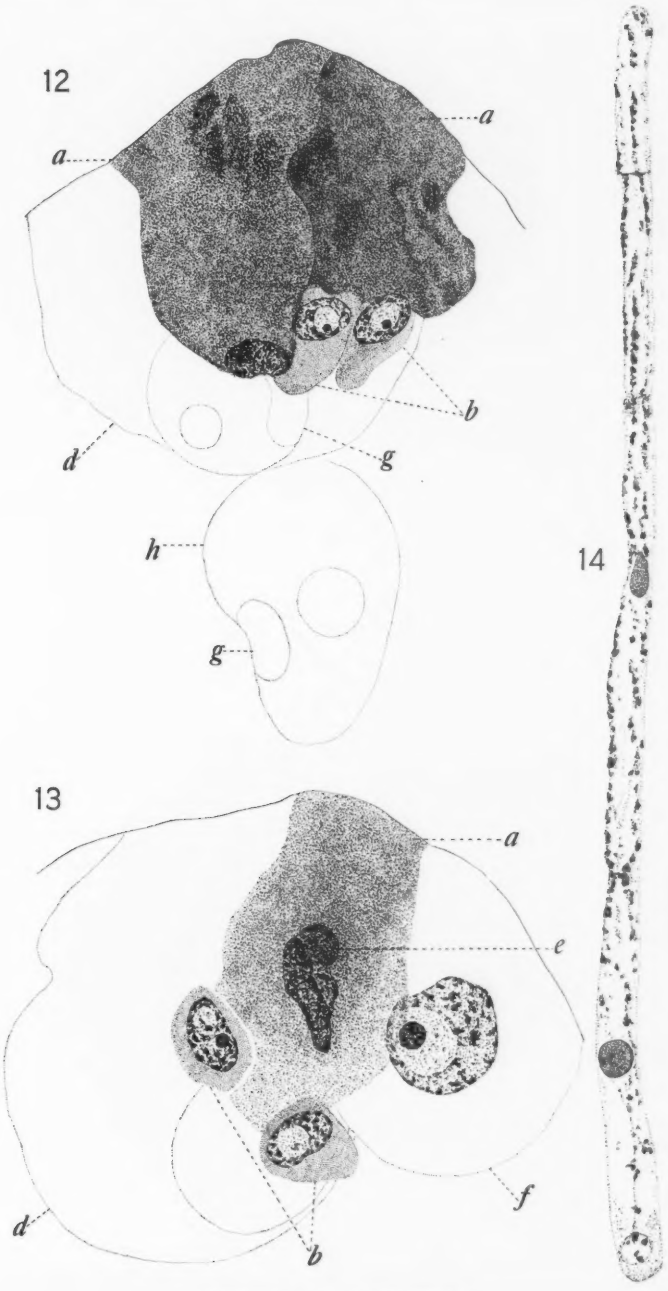
FIG. 14.—Elongated tube nucleus.



WYLIE on VALLISNERIA



WYLIE on VALLISNERIA



WYLIE on VALLISNERIA

LIFE HISTORY OF CEDRUS ATLANTICA

R. WILSON SMITH

(WITH PLATE XI)

Some years ago, through the kindness of the authorities of the Royal Botanic Gardens at Kew, the writer obtained ovules and stamens of *Cedrus atlantica* in various stages of development. A brief examination of these showed that much of the material was worthless on account of deficient pollination, but two interesting peculiarities invited further investigation. Frequently after an egg had begun to produce a proembryo, the ventral canal cell and neck cells of the archegonium were found to be still uninjured, and no trace of pollen tubes could be discovered in the nucellus or in the archegonium. Also a great many eggs, especially such as were apparently just ready for fertilization, contained from one to three small supernumerary nuclei. These conditions suggested the possibility either of parthenogenesis as described by SAXTON (**8**) for *Pinus Pinaster* on somewhat similar evidence, or of the fertilization of the egg nucleus by one of these supernumerary nuclei.

Sir DAVID PRAIN, Director of the Kew Gardens, very kindly supplied additional material, and at my request also had a number of ovulate cones protected with nets at the time of pollination, for the purpose of testing the hypothesis of parthenogenesis. The report of the netting experiment was as follows: "The cones protected during pollination time failed to develop and died. All the other female cones on the same tree were likewise arrested, so that the experiment, unfortunately, gave no result." In the meantime I had examined the additional material and found in it many pollen tubes and evidence of normal fertilization. The investigation thus lost most of its interest, but since no data in reference to the gametophyte generations of *Cedrus* have yet been published, the following account is given as a matter of record.

The material consists of very young staminate and ovulate cones, staminate cones collected at intervals for several weeks before time of pollination, and ovulate cones shortly after pollination and

at intervals from March until August. *Cedrus atlantica* furnishes a striking example of the indirect influence of pollination. Whilst an unpollinated cone atrophies and drops off the tree, a very small amount of pollen received into a cone suffices to stimulate it so that the ovuliferous scales develop fully and equally, and there is even a considerable enlargement of all the ovules.

The young cones, both staminate and ovulate, begin to form early in summer and continue to grow until autumn. Pollination takes place about the first of October. Previous to the shedding of the pollen, the microgametophyte advances one division further than it does in *Pinus*, consisting in *Cedrus* of two much flattened prothallial cells, and body, stalk, and tube cells (fig. 1). This condition, not that of *Pinus*, must be considered characteristic of the Abietineae, since it obtains in seven of the nine genera: *Cedrus*, *Larix*, *Picea*, *Abies*, *Tsuga*, *Pseudolarix* (MIYAKE **6**), and *Keteleeria* (HUTCHINSON **3**). *Pseudotsuga* (LAWSON **4**) is the only genus agreeing with *Pinus*. Sometimes in species of *Abies* (*A. balsamea* and *A. Veitchii*) the male gametophyte reaches its full complement of cells before the shedding of the pollen, the male cells frequently being already formed by the division of the body cell.

Two peculiarities were observed in the pollen: irregularity of development within each microsporangium, and long retention of the pollen after it had reached the mature condition. In all the stamens collected on the different dates (September 1, 9, 16, and 23), most of the pollen grains were mature, as in fig. 1; but in every sporangium there were also pollen grains in an earlier stage of development, namely, some with only the antheridial cell (fig. 2), others with generative cell and tube nucleus, and others in various stages of nuclear division. The prothallial cells were very difficult of recognition; they could be demonstrated only in a few favorable specimens of the earliest material at my disposal.

One feature which needs further investigation is the date when the pollen grains germinate. From the material it would appear that they remain quiescent without putting out a pollen tube from October until June. In ovulate cones collected in October, the pollen grains were quite numerous between the scales, but one was rarely seen within the micropyle. None of these had put out a

tube, nor was a tube found in any of the material collected from March to May, although the fixation was adequate to preserve the contents of the pollen in good condition. Later material, which contains evidence of pollen tubes in the nucellus, shows that there is none of the branching or irregularity of the pollen tubes that is so conspicuous a feature in *Pinus*. The tissues of the nucellus are rather loose, and the pollen tubes take a direct and very straight course. Some grains in contact with the nucellus evidently do not germinate (fig. 5, July 1), but still retain their contents.

As so generally among the Coniferae, the megaspore mother cells of *Cedrus* appear about the time of pollination. Only one mother cell appears in an ovule, deeply placed, and with about six or eight irregular layers of cells above it (figs. 3, 4). One side of the young integument is much longer than the other, and is folded inward so as to form a groove. In older ovules the nucellus is prolonged into a pointed protuberance extending far up into the micropyle, whose opening is lateral to the long axis of the ovule and turned toward the ovuliferous scale (fig. 5). Large glandular cavities, 2–4 in number, appear within the integument in the older ovules. These may help to give buoyancy to the seeds, which ultimately reach a length of more than a centimeter. In October material some of the megaspore mother cells are in the prophase of division, but later stages were wanting.

The embryo sac passes through the usual sequence of development. Archegonium initials first appear toward the end of May. The number of archegonia to an ovule is 4–6, usually 5. They are placed in an apical group converging toward the nucellus, and so close and crowded that often there is not room for the full growth of the jacket cells between them. Even in the best pollinated material, two or three archegonia of each group remain sterile. The neck of the young archegonium consists of a single tier of 2–4 cells (figs. 6, 7). It quickly becomes two or three tiers deep. The cells of the neck are very irregular in arrangement, the walls often running obliquely and the cells generally becoming multinucleate (fig. 8). In many of the older archegonia the neck becomes a heap of very small uninucleate cells by aggregation of the protoplasm around the numerous nuclei. Fertilization probably occurs

in the latter days of June. Entrance of the pollen tube into the archegonium and fusion of the sexual nuclei were not observed. Unfortunately the only material of these latter days of June was fixed in a mixture of alcohol and formalin, and is not suitable for cytological observations. Well preserved material of date July 1 contains young proembryos, and although in a few instances I could find what appeared like withered pollen tubes in the necks of the archegonia, in other and much more frequent cases the utmost care and search failed to discover any traces of pollen tubes. Notwithstanding this, I cannot believe this is a case of parthenogenesis; first, because better pollinated material produced a larger proportion of fertile archegonia, and second, because parthenogenesis fails to explain why so large a proportion of healthy normal eggs never pass beyond the "ready for fertilization" stage.

The occurrence of supernumerary nuclei in the protoplasm of the egg has already been mentioned. Similar nuclei have been seen in other Abietineae: in *Tsuga* (MURRILL **7**), *Abies* (MIYAKE **5**, HUTCHINSON **2**), *Pseudolarix* (MIYAKE **6**), etc. Only HUTCHINSON has studied the origin and behavior of these abnormal nuclei. He found that the ventral canal cell nucleus in *Abies balsamea* slips back into the egg and behaves as an egg nucleus, uniting with one of the male cells brought in by the pollen tube, and subsequently dividing into four free nuclei. Because of this unique case, and because of the segmentation of eggs in *Cedrus* apparently without the stimulus of fertilization, I have made a rather detailed study of the ventral canal cell. The apex of an archegonium cutting off the ventral canal cell is shown in fig. 9. It will be observed that the two nuclei are equal in size, and contain equal quantities of chromatin. Subsequently a cell wall appears and a definite and very persistent ventral canal cell is formed. Fig. 10 shows such a case. The ventral canal cell, still plump and healthy, retains its nucleus, although development of a proembryo is in progress; no supernumerary nucleus is present. In the case represented by fig. 11 the ventral canal cell nucleus is almost empty, and two large nuclei lie below it in the egg. In the adjacent section these two are seen not to be completely separated by a membrane. The supernumerary nuclei vary in size as well as in number. Usually they

are much smaller than the egg nucleus. In fig. 15, however, there are two of them, comparatively empty and much enlarged, and the ventral canal cell has disappeared. Fig. 12 may be regarded as a typical case, the ventral canal cell still retaining its nucleolus and much of its chromatin. In a number of cases I have been fortunate enough to catch the ventral canal cell nucleus in the act of discharging into the egg. No other interpretation is possible in such cases as figs. 13 and 14. Fig. 14 is particularly convincing, showing as it does how the streaming nuclear matter was unable to push forward one of the large proteid granules and flowed past it on either side. These supernumerary nuclei in *Cedrus atlantica* play no important part in the life history. They never approach the egg nucleus, and hence cannot stimulate it to division, and careful study of well fixed material fails to discover their capability of fertilization by a male cell. They are simply absorbed into an egg which appears to be already sufficiently well nourished.

The embryogeny agrees very closely with that of *Pinus* (figs. 10, 15, 16, 17). Two successive divisions of the egg nucleus take place near the middle of the egg, the four resulting nuclei passing to the base and there giving rise to four tiers of four cells each, the upper four not walled in on the upper side. The lower cells give rise to the embryos, of which, as in other Coniferae, but one comes to maturity. Probably also the rosette layer develops into abortive embryos, as discovered by BUCHHOLZ (1) in *Cedrus libani* and many other Abietineae, but no attempt was made to repeat his demonstration. The rosette cells enlarge considerably, but for a week at least remain undivided and uninucleate (fig. 14).

Summary

1. The ripe microspore contains two much reduced prothallial cells, and body, stalk, and tube cells.

2. There is a single deeply placed megaspore mother cell.

3. Pollination occurs about the first of October and the pollen grains then received into the ovulate cone probably do not send out tubes until the following June.

4. Pollination in the material available was so deficient as to suggest the possibility of parthenogenesis.

5. The number of archegonia is 4–6. There is a definite and very persistent ventral canal cell which frequently discharges part of its nuclear matter into the egg, thus giving rise to supernumerary nuclei in the latter.

6. Embryogeny agrees very closely with that in *Pinus*.

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EXPLANATION OF PLATE XI

Figs. 1, 2.—Microspores, September; ×402.

Figs. 3, 4.—Longitudinal and transverse sections of ovules and related structures: *o*, ovuliferous scale; *b*, bract; *t*, integument; October; fig. 3, ×80; fig. 4, ×39.

Fig. 5.—Longitudinal section of tip of ovule, showing micropyle and three archegonia; July 1; ×30.

Figs. 6, 7.—Tips of young archegonia; June 13; ×262.

Fig. 8.—Necks of three archegonia; July 1; ×262.

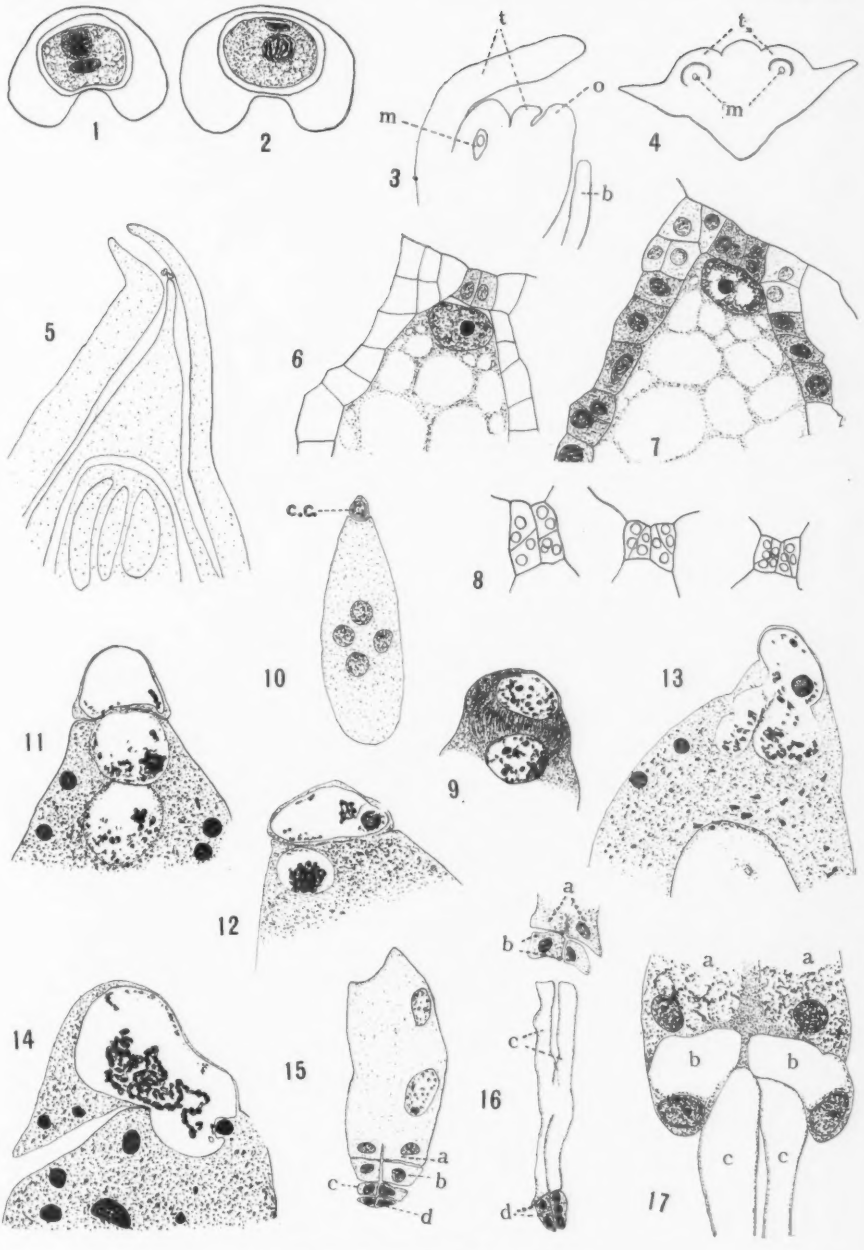
Fig. 9.—Apex of archegonium cutting off ventral canal cell; June 24; ×402.

Fig. 10.—Archegonium with proembryo and ventral canal cell; July 1; ×80.

Figs. 11, 12.—Tips of archegonia, showing supernumerary nuclei; July 1; ×402.

Figs. 13, 14.—Ventral canal cell nucleus discharging into egg; July 1; ×402.

Figs. 15–17.—Development of embryos: *a*, upper tier of cells; *b*, rosette tier; *c*, suspensor tier; *d*, embryo tier; fig. 15, July 4, ×97; fig. 16, July 6, ×97; fig. 17, July 8, ×237.



SMITH on CEDRUS ATLANTICA

BRIEFER ARTICLES

SIMPLE WEIGHING DEVICE FOR LARGE-SCALE TRANSPIRATION EXPERIMENTS

(WITH TWO FIGURES)

A number of different devices have been employed for weighing the large cans used as containers in many transpiration experiments. Most of these make use of some sort of overhead frame, from which the can is suspended for weighing. For use with very large herbaceous plants, or with trees ten feet high or more, such devices are unsatisfactory, since the ropes or chains used to lift the cans disturb the foliage.

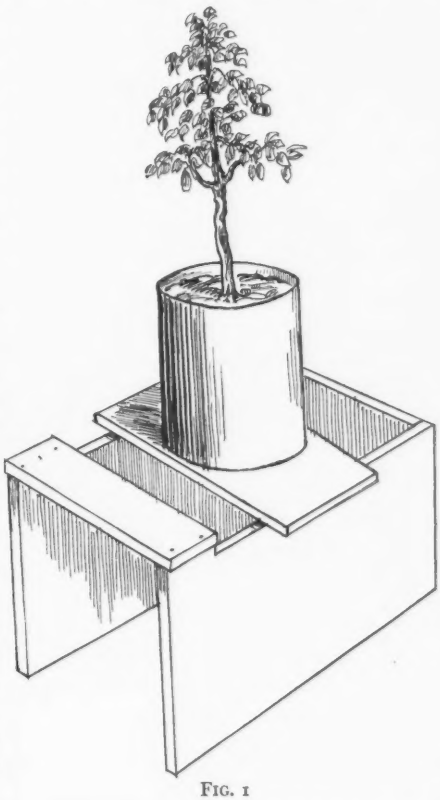


FIG. 1

In such cases a method of lifting the cans from beneath may be used to advantage. The cans are set on boards supported on wooden structures similar to that shown in fig. 1, or for permanent installations parallel concrete piers may be built for supporting the boards. A good platform scale of sufficient capacity is used for weighing the cans. If the scale is provided with wheels, these are removed and the scale is mounted on a steel lifting truck of the type commonly used in warehouses. Such a truck has a vertical

lift of about two inches, and several hundred pounds can be lifted with it without much effort. Fig. 2 shows a scale thus mounted. To weigh the containers, the truck is backed into the open front of the wooden support (or between the concrete piers if these are used). These structures must be built of such a height that the cross-board will allow the platform of the scale to pass under it without touching. When the platform of the scale is under the cross-board the truck is lifted. The cross-board with the container and plant now rests entirely on the scale, and the weight may be ascertained. The truck is lowered, wheeled out of the support, and on to the next.

FIG. 2

The weighing can be done in a much shorter time than with overhead devices where hooks must be attached to the containers for lifting them. This is an item of importance when many containers are used. The plants are weighed without jarring or disturbing the foliage. The cross-boards should be made strong enough to support the weight of the containers without sagging, and should be waterproofed thoroughly to prevent decay and increase of weight by the absorption of moisture.

The apparatus is inexpensive, is portable, and does not deteriorate rapidly. It has the further advantage of requiring nothing which cannot be secured in the market at all times.—CARL D. LA RUE, *Ann Arbor, Michigan.*

A CORRECTION

In Miss EDA M. ROUND'S recent paper on "*Annularia* with *Paleostachya* fruit" (BOT. GAZ. **73**:326–328. 1922), she quotes a passage from my *Studies in fossil botany* somewhat inaccurately, so as to convey a wrong impression. The passage is quoted as follows: "In all cases where fructifications have been referred to *Annularia* . . . . they have been proved to be of the *Calamostachys* type." What I actually said was: "In all cases where fructifications have been referred to *Annularia* or *Asterophyllites*, they have so far proved to be of the *Calamostachys* type; it is therefore evident that no constant correlation between reproductive and vegetative characters can be traced" (3d ed., vol. I, p. 67). This gives quite a different impression from Miss ROUND'S rendering of the passage. There was obviously no "contention" on my part, and consequently nothing to "refute" (Miss ROUND, p. 328). I was simply stating the evidence as it appeared to present itself at the time.

Miss ROUND'S observation of a fruit inferred to be *Paleostachya* in connection with a shoot of an *Annularia* is an interesting addition to the evidence, but in no way refutes any contention of mine.—D. H. SCOTT.

CURRENT LITERATURE

BOOK REVIEWS

The broad sclerophyll vegetation of California

No type of vegetation has been associated more closely with the early development of our civilization than that characterized by broad, thick, evergreen leaves, such as are borne by the laurel, olive, holly, and evergreen oak that grew upon the hillsides of the Mediterranean region of Europe and Asia. Possibly because of this early association with civilized man and the consequent devastation, there have been few if any satisfactory ecological studies of such vegetation, even in the old world. The present volume,[1] therefore, is the first adequate attempt at an explanation of similar forests and scrub lands found upon the Pacific coast of North America. The region of vegetation, as here defined by COOPER, centers in Ventura and Santa Barbara Counties of California, or that part of Southern California lying between the deserts and the Pacific Ocean. From this center it extends, with decreasing luxuriance, into northern Lower California, southern Oregon, and spreads itself along the eastern slopes of the Sierras that lie within the same latitude.

The climatic limit appears to include that part of western North America where the annual precipitation lies between 10–30 inches, and where less than 20 per cent of this rain falls within the summer months. The region is also characterized by more moderate temperature extremes than those of the adjacent desert at the east and south, or of the conifer forest region at the north. Two main divisions of the broad sclerophyll vegetation are recognized, the broad sclerophyll forest and the chaparral formation, while the latter is separated into two subdivisions, the climax chaparral and the conifer forest chaparral. Northward the forest is the more important, especially in the Coast Ranges, while southward the chaparral becomes more and more predominant.

The broad sclerophyll forest is dominated by relatively few species of trees, mainly sclerophyllous evergreens, but with a mixture of less than one-third deciduous species. Among the former, *Myrica californica*, *Castanopsis chrysophylla*, *Quercus agrifolia*, *Q. chrysolepis*, *Q. Engelmannii*, *Q. Wislizeni*, *Pasania densiflora*, *Umbellularia californica*, and *Arbutus Menziesii* are the chief species, while the representatives of the latter are *Quercus Kelloggii*, *Q. lobata*, *Acer macrophyllum*, and *Aesculus californica*. Several distinct associations are recognized from the constancy of the dominance of one or more of the preceding

[1] COOPER, W. S., The broad sclerophyll vegetation of California. An ecological study of the chaparral and its related communities. 8vo. pp. 124. *pls. 21. figs. 43.* Carn. Inst. Wash. Publ. 319. 1922.

species. In its transition to the *Pseudotsuga* and *Sequoia* forests, the broad sclerophyll forest associations often form an understory to the taller conifers.

The climax chaparral is more widely spread than the forest just mentioned, with which it intergrades. It covers practically all of the southern Coast Ranges, together with the mountains of Southern California and northern Lower California. COOPER also finds evidence that certain extensive areas now covered by grasses and half-shrubs are climatically and potentially chaparral regions. The species are here more numerous, and include *Adenostoma fasciculata*, *Heteromeles arbutifolia*, *Rhus* (3 spp.), *Rhamnus* (2 spp.), *Ceanothus* (10 spp.), *Arctostaphylos* (9 spp.), *Cercocarpus betulaefolius*, and several less important forms.

The range of the conifer forest chaparral is found to be nearly coextensive with that of the montane conifer forest in the Sierras and Coast Ranges of California and Oregon. Its lower limit seems to coincide with that of *Pinus ponderosa*, and in its composition are included many of the same genera as are found in the climax chaparral, although often represented by different species. There is also an admixture of deciduous shrubs, such as deciduous species of *Quercus*, *Ceanothus*, and *Prunus*.

From an intensive study of a type locality in the Palo Alto region, where temperature, rainfall, soil moisture, evaporation, and other factors were carefully measured for a period of nearly two years, many exact data are accumulated. From these, from quadrat studies, and from other material, COOPER concludes that the controlling factors and the fundamental distinguishing difference between the two broad sclerophyll climaxes are the water balance and its variations, and that while the forest dominates no extensive region, nevertheless it must be considered a climax, representing a degree of mesophytism between the chaparral and the conifer forest, and showing a tendency to gain dominance over the chaparral. The climax nature of the larger portion of the chaparral association is also indicated by its dominance, its stability, its occurrence on diverse sites as to soil and topography, and by its adjustment to climate. There is also found direct evidence of climax nature from a study of the primary and secondary successions themselves, leading up to the establishment of a permanent community in the chaparral association. Proof is furnished that the chaparral formerly covered large areas now held by grassland associations. The agencies which have led to the invasion of the grasses are indicated as clearing, cultivation, and more particularly fire.

Some detailed studies of a few of the root systems, an analysis of the leaf sizes according to RAUNKIAER's methods, and an examination of the internal structure of the leaves of 26 representative species, together with an annotated list of nearly 100 species, and a bibliography of 90 titles occupy the final pages of the book.

The organization of the material and its clear presentation, the abundance of quantitative data, the careful presentation of the dynamics of the situation, and the importance of the conclusions, all go to mark this as one of the most

notable contributions to ecological science that has appeared during the past few years. The maps and numerous good photographs, well reproduced, greatly aid in adding interest to the text.—GEO. D. FULLER.

Roots of crop plants

The botanical world is again indebted to WEAVER[2] for a notable contribution to our knowledge of root development. The present volume comes as a continuation of *Root development in the grassland formation*, previously reviewed in this journal,[3] since the studies were made in the same grassland region, the principal stations being at Peru and Lincoln, Nebraska; Phillipsburg, Kansas; and Burlington, Colorado. These stations have respectively 33, 28, 23, and 17 inches of annual precipitation, and are representative respectively of the subclimax prairie, true prairie, mixed prairie, and pure short grass vegetation. For each of the stations such factors as temperature, soil composition, and soil moisture are given in detail, the last in terms of water content in excess of hygroscopic coefficient. The experiments reported were carried on with oats, wheat, barley, corn, and potatoes during the seasons of 1919, 1920, and 1921, and consistent results were obtained during each of the three summers. These crops were grown under conditions closely comparable to those of ordinary field tillage, and their roots excavated and studied at various intervals throughout the growing season up to the full maturity of the crop. The methods of exacavation and representation are simple and the same as those previously used, but such as require much skillful labor and patience, which has been freely given by the investigators with a resulting abundance of significant data. The system of plotting the root systems upon paper ruled to a uniform scale of square feet is to be commended for the ease with which comparisons may be made.

As in previous studies, the first most striking root character is the depth to which the roots extend, and here there is a notable correlation between the roots of native vegetation and those of crop plants. In the true prairie 65 per cent of the native plants were found previously to possess roots reaching to a depth greater than 5 feet, and in the mixed prairie 44 per cent of the species attained such a penetration, while in the short grass plain only 12 per cent reached that depth. During 1920 and 1921 crop plants reached the following average depths at Lincoln in true prairie, at Phillipsburg in mixed prairie, and at Burlington in short grass: oats 4.8, 5.8, and 2.6 feet; wheat 4.6, 5.1, and 2.6 feet; barley 5.0, 6.3, and 2.7 feet respectively. Working depths for oats were 2.7, 3.1, and 2.4 feet; wheat 3.0, 2.9, and 2.4 feet; and barley 3.1, 3.2, and 2.2 feet respectively. Using the root development in the true prairie as unity, the relative working depths of roots in prairie, mixed prairie, and short grass

[2] WEAVER, J. E., JEAN, FRANK C., and CRIST, J. W., Development and activities of roots of crop plants. Carnegie Inst. Wash. Publ. 316: pp. 117. *pls. 14. figs. 42.* 1922.

[3] BOT. GAZ. **73**:412–413. 1922.

were rye 100:92:69; oats 100:95:75; winter wheat 100:93:61. Maximum depths of rye were 100:90:65; oats 100:94:77; and winter wheat 100:80:51. It is highly probable that penetration in the deep loess soil of the prairies and plains is somewhat greater than might be expected elsewhere, and yet two of the same workers,[4] investigating conditions in portions of the plains where a layer of so-called "hardpan" exists to a depth of 1.4–3 feet below the surface, found that the roots of both native and crop plants often penetrated to a depth of 5 or 6 feet. The controlling factor here appears to be the penetration of moisture and not the hardpan layer. No modifications of root habit were apparent in the hardpan layer, which here varies in thickness from 8 inches to 1.5 feet, and while in some localities it appears to be due largely to the calcareous nature of the soil, in others the cementing effect of colloidal clay is also in evidence.

Throughout these studies the root systems were very extensive in relation to the aerial parts, and abounded in branches which largely increased their absorbing area. All cereals, including corn (*Zea*), showed a root system in which there was a definite group of horizontal spreading roots superficially placed in the first foot of the soil, and a second group of more vertical roots reaching depths of 5–7 feet. The potato differed in the early development of horizontal roots which subsequently turned rather abruptly downward. Roots of isolated plants were decidedly different in distribution from those of plants growing with the ordinary spacing of crop production. Oats and corn seemed to reduce soil moisture to a greater extent than did other crops. Experiments conducted with containers provided with wax seals at various levels and with nitrogenous fertilizers added at various depths showed that where roots came in contact with a fertilized layer they developed more abundantly, branched more profusely, and were apparently retarded from normal penetration into the soil below. Fertilizing the surface soil in semi-arid regions, therefore, would seem to be somewhat detrimental to crop production by hindering root penetration.

The volume is well illustrated with carefully made drawings of the root systems, and by numerous photographs well reproduced, reflecting upon the publishers as well as the investigators. Many of the data regarding environmental conditions and root features are arranged in tables convenient for reference, and the organization of material is simple and logical.—GEO. D. FULLER.

Injury, recovery, and death

During the last fifteen years OSTERHOUT has devoted himself to a study of injury, recovery, and death, using mainly electrical conductivity methods for detecting changes in permeability of tissues. He has now summarized the work done as one of the Lippincott series of monographs of experimental

[4] WEAVER, J. E., and CRIST, J. W., Relation of hardpan to root penetration in the great plains. Ecology 3:237–249. *figs.* 5. 1922.

biology.[5] The six chapters deal with the methods of measuring electrical conductivity, the mechanism of the process of death, injury and recovery, antagonism, anaesthesia, and conductivity and permeability.

OSTERHOUT believes that electrical conductivity determinations afford a reliable means of measuring the vitality of living tissues. Toxic substances fall into two classes: those which cause immediate decrease of resistance; and those which cause an initial increase in resistance, followed by a decrease. To account for the changes in resistance, he assumes that resistance is mainly due to some surface in the protoplasm, formed as one step in a series of catenated chemical life reactions. If this series of reactions is modified so as to cause increase of the hypothetical substance, resistance increases; and if the reactions are changed in the direction of less production, resistance decreases. This series of catenated reactions is looked upon as irreversible; and injury from reduced resistance, and the recovery from injury, are supposed to depend on the relative speed of formation and decomposition of the substance upon which normal resistance depends. In general he finds that the substances which cause immediate decrease of resistance are antagonized by those which cause an initial increase in resistance. Thus NaCl is antagonized by $CaCl_2$, and by any other substance causing similar initial increase. The mechanism of antagonism is considered not necessarily the same in all cases. Anaesthesia is marked by a notable and reversible increase in resistance, which means decrease in permeability. Such decreases in permeability would hinder transmission of stimuli in so far as this depends on ionic migration through the tissues.

The author has developed an elaborate mathematical treatment of the quantitative data of injury and recovery. By use of suitable constants and formulas, curves may be calculated which approximate the experimental data. The reviewer feels that the mathematical treatment does not greatly strengthen the hypotheses and assumptions made by the author regarding the nature of injury, recovery, and the process of death. The main value of the formulas lies in their use to predict the behavior of the tissue of the particular plants for which the constants have been worked out, under conditions which have already been evaluated. This means that their value is quite restricted. The book contains mainly material that has already been published in numerous brief papers.—C. A. SHULL.

Climates of the continents

KENDREW[6] has published a book on climates, the purpose being, as stated in the introduction, to make available in the English language some adequate description of the actual climates of the countries of the earth considered region-

[5] OSTERHOUT, W. J. V., Injury, recovery, and death, in relation to conductivity and permeability. pp. 259. Philadelphia and London: J. B. Lippincott Co. 1922.

[6] KENDREW, W. G., Climates of the continents. 12mo. pp. xvi+387. *figs. 149.* New York: Oxford Univ. Press. 1922. $7.00.

ally. The information has been compiled from a variety of sources, and the results are somewhat unequally satisfactory for the various continents, largely on account of the inadequate nature of some of the data. The separate treatment of the main features of the climate of each region is the more satisfactory, since each is considered in its relation to the greater units of the globe. The climatic maps for the continents and portions of continents give every evidence of accuracy, and are sufficiently free from minor details to be read easily. They should prove an invaluable aid to studies of geobotany and geography, and the book will be indispensable to all ecologists and to botanists interested in problems of distribution in which climatic factors are involved.—GEO. D. FULLER.

MINOR NOTICES

Cactaceae.—The third volume of the monograph of Cactaceae by BRITTON and ROSE[7] has just appeared. The preceding volumes were reviewed in this journal.[8] In fullness of description and wealth of illustration it leaves nothing to be desired, the colored plates being especially noteworthy. The present volume includes three subtribes of Cereeae. The subtribe Echinocereanae includes six genera, three of which (*Austrocactus*, *Chamacereus*, *Lobivia*) are new. In this subtribe 20 new species are also described. The subtribe Echinocactanae includes 28 genera, 7 new species being described. The disintegration of *Echinocactus* is most noteworthy, most of the 17 new genera of the subtribe being segregated from it, leaving the old genus with only 9 species. The new genera are *Denmoza*, *Copiapoa*, *Toumeya*, *Neoporteria*, *Oroya*, *Arequipa*, *Matucana*, *Hamatocactus*, *Strombocactus*, *Ferocactus*, *Echinomastus*, *Homalocephala*, *Hickenia*, *Frailea*, *Mila*, *Sclerocactus*, and *Utahia*. The subtribe Cactanae includes two genera, in which five new species are described.—J. M. C.

Ferns of Bombay.—BLATTER and D'ALMEIDA[9] have published an interesting little manual of ferns of the "Bombay Presidency." It seems that this group has been much neglected by local botanists. The introduction contains a sufficient account of the structure and general features of ferns to enable the amateur to use the manual in identifying them. There are 141 species described, 130 of which belong to the Polypodiaceae, the 5 other families included containing only 11 species. The species of Polypodiaceae are distributed among 46 genera, the largest being *Adiantum* (14 spp.); 24 of the genera contain only a single species each.—J. M. C.

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[7] BRITTON, N. L., and ROSE, J. N., The Cactaceae. Vol. III. Publ. Carnegie Inst. no. 248. pp. vii+255. *pls. 24. figs. 250.* 1922.

[8] BOT. GAZ. **68**:391. 1919; **70**:404. 1920.

[9] BLATTER, S. J., and D'ALMEIDA, J. F., The ferns of Bombay. pp. viii+228. *pls. 17. figs. 43.* Bombay: D. B. Taraporevala Sons & Co. 1922.

NOTES FOR STUDENTS

Taxonomic notes.—BRAINERD[10] has published an account of the violets of North America, which is the result of over 25 years of intensive study, both in the field and in cultures. He was early led to believe that there is extensive crossing in nature, and that many so-called species are really hybrids. In the present publication he describes and illustrates 75 species, followed by a list of hybrids which will be discussed in a later contribution. BRAINERD has also studied *Rubus* in the same way, and in an earlier publication states that he had become "painfully aware of the confusion that had arisen from publication of scores of obscure and illegitimate species in these two genera based on scanty or immature material."

PEYRONAL[11] has described the following new genera of fungi from the Piedmont region of Italy: *Alysisporium* (Sphaerioidaceae) and *Chaetalysis* (Leptostromataceae).

CHODAT[12] in connection with studies of the algae of the Grand St. Bernard region, has described, in addition to a number of new species, the following new genera: *Cyanospira*, *Chrysosphaera*, *Bernardinella*, and *Pseudomallomonas*.

Miss LYLE[13] has described a new genus (*Antithamnionella*) of Rhodophyceae. It is an epiphytic form which she discovered in Guernsey in 1921. She says it is difficult to account for the presence of this alga in British waters, "the only plants approaching it in appearance or structure belonging to South Africa and Cape Horn."

SARGENT[14] has recently described 25 new species of *Crataegus*, which seems to be a most prolific genus with the new method of attack.

BAILEY[15] has made a critical taxonomic study of the cultivated species of *Brassica*. The results are obtained from long experimental study, using wild specimens as well as those in cultivation in different countries. Special attention is given to the Chinese and Japanese forms, "since they seem to be little understood botanically and are beginning to appear freely in North America." There is a full presentation of the problem involved, the limitations of genera, and the historic species, followed by a detailed description and discussion of

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[10] BRAINERD, EZRA, Violets of North America. Vermont Agric. Exp. Sta. Bull. 224. pp. 172. *pls. 85*. 1921.

[11] PEYRONEL, B., Champignons nouveaux des vallées vaudoises du Piémont. Bull. Soc. Mycol. France **38**:140–143. *pls. 4, 5*. 1922.

[12] CHODAT, R., Algues de la région du Grand St. Bernard. Bull. Soc. Bot. Genève 293–305. 1921.

[13] LYLE, LILIAN, *Antithamnionella*, a new genus of Algae. Jour. Botany **60**:346–350. *figs. 6*. 1922.

[14] SARGENT, C. S., Notes on North American trees. X. New species and varieties of *Crataegus*. Jour. Arnold Arboretum **3**:182–207. 1922.

[15] BAILEY, L. H., Brassicae cultorum. Gentes Herbarum **1**:53–108. *figs. 18–49*. 1922.

the 19 species and 11 varieties recognized, including 5 new species and 6 new varieties.

YUNCKER[16] has published a revision of the species of *Custuca* known to occur in South America and the Galapagos Islands. He recognizes 38 species, 7 of which are described as new. He has also segregated 5 new varieties. Only 8 of these species are found in North America or the West Indies.

NAKAI,[17] in continuation of his *Flora sylvatica* of Korea, has presented the Sarmentaceae, Tiliaceae, and Elaeocarpaceae, including 17 species, each of which is illustrated by a plate. The largest genus is *Tilia*, with 9 species, 6 of which are referred to NAKAI. The accounts are very detailed, and mostly in Japanese, but all the specific descriptions are published also in English.

An illustration of the wealth of material awaiting discovery in Africa is given by a recent contribution by BOLUS,[18] in which he describes 31 new species of *Mesembrianthemum*, and 10 additional species in other genera.

SYDOW and PETRAK,[19] in publishing a list of fungi from North America, especially from the northwestern states, have described 28 new species representing 26 genera, and have established *Clypeothecium* (Sphaeriaceae), *Entoleuca* and *Xyloschizon* (Hypodermeae), *Rhabdocline* (Phacidiaceae), *Xenodomus* (Sphaeropsideae), *Lagynodella* (Sphaeropsideae), *Lecanosticta* (Excipulaceae), *Rhabdogloeum* and *Microgloeum* (Melanconiaceae) as new genera.

SYDOW[20] has proposed the following new genera of Uredineae: *Persooniella*, *Schroeterella*, *Trailia*, *Poliomella*, *Haplotelium*, *Argomycetella*, and *Poliotelium*.

PETRAK,[21] in presenting a list of the fungus flora of the southern Alps, has established *Phomopsina* as a new genus of Sphaeropsideae. The list also includes 12 new species in various genera.—J. M. C.

Respiration and photosynthesis.—Phenomena involving gaseous interchange continue to stimulate refinements of technique as well as accumulation of data by means both old and new. OSTERHOUT[22] and his students have been employing a device consisting of two vessels in a closed system equipped for circulation of the contained air. One of these vessels contains the organism

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[16] YUNCKER, T. G., Revision of the South American species of *Cuscuta*. Amer. Jour. Bot. **9**:557–575. 1922; **10**:1–17. *pls. 1–5*. 1923.

[17] NAKAI, T., Flora sylvatica Koreana. XII. pp. 63. *pls. 17*. Seoul, Corea. 1922.

[18] BOLUS, H. M. L., Novitates Africanae. Ann. Bolus Herb. **3**:123–146. 1922.

[19] SYDOW, H., and PETRAK, F., Ein Beitrag zur Kenntnis der Pilzflora Nordamerikas, insbesondere der nordwestlichen Staaten. Ann. Mycologici **20**:178–218. 1922.

[20] SYDOW, H., Weitere Mitteilungen zur Umgrenzung der Gattungen bei den Uredineen. Ann. Mycologici **20**:109–125. 1922.

[21] PETRAK, F., Beiträge zur Kenntnis der Pilzflora der südlichen Alpenländer und Norditaliens. Ann. Mycologici **20**:126–159. 1922.

[22] OSTERHOUT, W. J. V. Jour. Gen. Phys. **1**:17 and 171. 1918.

to be studied, in culture solution, while the other contains an indicator solution. Respiration rates under various conditions are measured by the time required to produce an arbitrary amount of color change. Studies upon respiration of bacteria, fungi, angiosperms, and animals as affected by organic reagents ("anaesthetics"), inorganic salts ("antagonism" studies), and variations in hydrogen ion content have been made. In general the higher concentrations of organic reagents used (ether, etc.) produce in plants an increase of CO_2 evolved, followed by a decrease, while in the animals studied this increase is preceded by an abrupt drop. Aside from a brief note on the inhibition of ether effects by NaCl in *Bacillus subtilis*, a case referred to as antagonism, this latter phenomenon has been studied in the case of chlorides of K, Na, Mg, and Ca. The least disturbance of respiration is shown by couples containing either $CaCl_2$ or $MgCl_2$, and one of the monovalent salts. The hydrogen ion experiments indicate that respiration in *Penicillium chrysogenum* is not affected by P_H values from 4.0 to 8.0, but at an alkalinity of P_H 8.80 undergoes a reversible drop. At an acid P_H 2.65 there is a temporary rise, and at P_H 1.10–1.95 a rise of 20 per cent, followed by an irreversible drop. It is safe to say that physiologists will be grateful when OSTERHOUT becomes free to turn the facilities and energies at his disposal from the work of reconnaissance to the investigation of some of the fundamental leads that have only been touched upon.

WILMOTT[23] has devised an improvement of the erstwhile disreputable bubble-counting device for measuring gas release from submerged plants. The gas is discharged as bubbles of uniform size by a tapered bent glass nozzle via an inner cup of distilled water, sealed off from the culture medium in which the plant is growing. By means of this apparatus sources of error due to initial diffusion effects of carbon dioxide and oxygen have been studied and largely eliminated. It has also been determined that increase of photosynthesis in presence of acids may be due to increased liberation of CO_2 from calcareous incrustations or solutes, while no evidence is found for assuming that water plants of themselves are capable of decomposing bicarbonates in their culture solutions.

BRIGGS[24] has been able to secure control of the earliest stages of greening and the beginnings of photosynthesis in seedlings. He uses an atmosphere of hydrogen supplied with carbon dioxide and removes oxygen as fast as it is formed. He finds that photosynthetic activity lags behind chlorophyll formation, and considers that the limiting factor is internal, but not based upon the amount of chlorophyll present. It is important to note that he considers WILLSTÄTTER's failure to agree with Miss IRVING, that young fully green leaves might show no activity, to be due to the use of leaves significantly older than those used by Miss IRVING. BRIGGS also gives good reason for the provisional analysis of photosynthesis into diffusion, photochemical, and chemical stages.

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[23] WILMOTT, A. J., Proc. Roy. Soc. London B **92**:304. 1921.

[24] BRIGGS, G. E., Proc. Roy. Soc. London B **91**:249. 1920.

In part I of a study by KIDD, WEST, and BRIGGS[25] these authors present comparative respiration data on whole plants and plant parts throughout the life history of a field crop of *Helianthus annuus*. Their results show a steady decrease in respiration of the plant and its parts with increasing age. There are also consistent local differences within the plant, respiration rates decreasing in the following sequence: inflorescence, stem apex, leaves, stem. The conception and laborious execution of the work here begun promise results of very great significance.—P. B. SEARS.

Soil moisture.—Seeking to apply the results of ecological investigations to the solution of problems of irrigation in Oregon, POWERS[26] has examined the relation between the moisture capacity of soil under field conditions, the wilting point of crops in the field, and the results obtained by irrigation at the various degrees of drying out. The practical results show that potatoes require more moisture for the best returns than does clover. The grower following the results of this experiment will know at just what degree of moisture irrigation water should be added to his field to produce best returns from these crops, and also the quantity of water that may be efficiently utilized. This latter varies from 0.5 inch in coarse sand and 1 inch in fine sand, to 1.75 inches in loam and 3–4 inches in peaty soils.

In plotting the results and showing the variation of hygroscopic coefficient, wilting point in the field, and usable water capacity, the author demonstrates that the wilting point in the field varies considerably for different plants, and he appears to assume, quite without any experimental evidence, that there is a similar variation in the wilting coefficient. Hence while we may admit the validity of his conclusion that "the wilting point varies more between different crops than has commonly been supposed when judged by field and tank studies of crop appearance, soil moisture, and yields of dry matter," his data do not justify the conclusions regarding variations in wilting coefficient until it be shown that "wilting point in the field" and wilting coefficient coincide or vary together.

That temporary wilting varies widely not only between different species of plants but at different temperatures, and even while soil moisture is decidedly high, has long been recognized, and is clearly demonstrated by recent experiments by PERALTA,[27] which show the difference in yields of lettuce with different amounts of water supplied regularly by auto-irrigations and irregularly by hand watering. A decided advantage appears in every instance in favor of a constant water supply, the increase in leaf area being more striking than that of dry weight.

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[25] KIDD, F., WEST, C., and BRIGGS, G. E., Proc. Roy. Soc. London B **92**:368. 1921.

[26] POWERS, W. L., Field moisture capacity and wilting point of soils. Soil Sci. **14**:159–165. *fig. 1.* 1922.

[27] PERALTA, FERNANDO DE, The control of soil moisture by means of auto-irrigators. Philippine Agriculturalist **10**:467–477. *figs. 3.* 1922.

Very interesting data as to temporary wilting of plants in soils with different amounts of moisture were obtained. For example, plants in soil supplied with water against a pressure of 40 cm. of mercury began to wilt at about 27° C., against 20 cm. of mercury at 28° C., against 10 cm. of mercury at 29° C., against 5 cm. of mercury at 30.7° C., and against 0 cm. mercury were still unwilted at 31.5° C. The constant water supply was effected by the use of the Livingston porous cup auto-irrigators.—GEO. D. FULLER.

Aluminum toxicity.—The possibility that the stalk and root rot of corn is associated with a high aluminum and iron deposit at the nodes has led ARNDT[28] to investigate the effects of aluminum and iron on the growth of corn. By the use of nutrient solutions he attempted to determine whether toxic concentrations of aluminum and iron will produce pathological conditions similar to those caused by parasitic organisms. Two solutions, "*A*" and "*H*," were used. The first corresponds to the type I—R_2S_3 solution recommended by the committee on salt requirements of representative agricultural plants. Solution "*H*" is a modified form of HARTWELL and PEMBER'S[29] solution. Varying concentrations of acids, salts of aluminum and ferrous and ferric iron were added. Solution and sand cultures were used, and the corn was grown from twenty-five to forty days.

ARNDT concludes that in his "*A*" solution the aluminum salts are readily precipitated, and the toxicity is due to the H+-ion concentration. In solution "*H*," however, toxicity is attributed to the aluminum and iron. The toxicity of the salts is said to be determined by the composition of the nutrient solutions. Some doubt may be expressed as to whether pathological conditions analogous to the stalk and root rot of corn can be produced within forty days of growth. Photographs accompanying the discussion do not indicate that the corn was sufficiently large for a quantitative determination of the aluminum and iron content of the nodes.

A most important contribution to our knowledge of aluminum as it affects the growth of plants is one by STOKLASA.[30] The experiments deal with the influences aluminum, along with iron and manganese, exerts on the growth of a large number of well known European plants. The author considers aluminum a necessary mineral nutrient for hydrophytic plants. He finds that the formation of colloids is greatly facilitated by the presence of aluminum, and much importance is attached to this element as an ash constituent. Quantitative comparisons and estimations have covered several years of experimental work. —J. C. IRELAND.

[28] ARNDT, CHARLES HOMER, The growth of field corn as affected by iron and aluminum salts. Amer. Jour. Bot. **9**:47–71. 1922.

[29] HARTWELL, B. L., and PEMBER, F. R., The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. Soil Sci. **6**:259–277. 1918.

[30] STOKLASA, JULIUS, Über die Verbreitung des Aluminiums in der Natur und seine Bedeutung beim Bau- und Betriebsstoffwechsel der Pflanzen. 8vo. pp. x+500. Jena: Fischer. 1922.

Clamp connections in an Ascomycete.—The recent work of KNIEP and BENSAUDE has shown conclusively that the clamp connections common in Basidiomycetes are formed in connection with the conjugate division of paired nuclei, and both workers have insisted that these structures are homologous with the croziers formed preceding the formation of asci. There has been no unquestioned report of the occurrence of clamp connections in the mycelium of an Ascomycete, however, until the MOREAUS,[31] in a recent note, have reported finding them on the walls of the ascogenous hyphae of the lichen *Parmelia Acetabulum*. These clamp connections occur, just as in the great majority of the Basidiomycetes in which they have been found, in connection with a binucleate mycelium, the asci arising as the terminal cells of chains of binucleate cells, provided with clamp connections at their septa. The authors' description of crozier formation differs from the accounts which have been given previously. The terminal cell develops a protuberance on one side near the center of the cell. This protuberance grows downward, forming a beak, and is cut off by a wall at its base. At the same time a septum develops in the terminal cell, dividing it into two cells just below the point of origin of the beak, forming a crozier exactly as the clamp connection is formed in Basidiomycetes.

According to this interpretation of the development of the crozier, the so-called "terminal cell" of previous writers is in reality a lateral outgrowth, the beak; the so-called "penultimate cell," developing into an ascus, is in reality the terminal cell; and the so-called "antepenultimate cell" is in reality the penultimate cell. As in other croziers which have been described, the beak may or may not fuse with the penultimate cell.

This discovery, if verified, will form another important link in the ever growing chain of evidence pointing toward the close phylogenetic connection between the Ascomycetes and the Basidiomycetes.—G. W. MARTIN.

Plankton in the Irish Sea.—HERDMAN[32] has published a summary of the results of an investigation of the plankton of the Irish Sea, carried on continuously during fifteen years. So far as the phytoplankton is concerned, the following statistics are of interest. The spring phytoplankton maximum ranges from March to June, and is composed chiefly of diatoms. The maximum "haul" of diatoms ranged from less than one million in 1907 to about 206 millions in 1913. A secondary less conspicuous maximum ranges from September to November. During the time of the diatom maximum in spring, the organisms are more evenly distributed over the sea and downward through the layers than at other times of the year.

It is thought probable that the vernal increase in phytoplankton, one of the great phenomena of the ocean, depends primarily upon the rapid increase

[31] MOREAU, M. and MME. FERNAND, Le mycelium a boucles chez les Ascomycetes. Compt. Rend. Acad. Sci. **174**:1072–1074. 1922.

[32] HERDMAN, SIR W. A., Summary of results of continuous investigation of the plankton of the Irish Sea during fifteen years. Jour. Linn. Soc. Bot. **46**:141–170. *pl. 7*. 1922.

in the amount of solar energy which accompanies the lengthening days of early spring, especially about the time of the vernal equinox. "Thus general meteorological conditions are linked up with the commercial fisheries of several years ahead." The author thinks that the diatom maximum in spring no doubt is aided by the winter increase of carbon dioxide and other food materials in the sea. The rapid disappearance of the diatoms after the maximum is thought to be due probably to some toxic effect upon the water caused by their metabolism in dense crowds.—J. M. C.

Formation and utilization of fats.—A good review of what is known and what is yet to be discovered in these two "symmetrical" plant processes is presented by TERROINE.[33] No doubt is left in the mind of the reader that the fats are formed from carbohydrates, and upon germination of the seeds are converted into carbohydrates again. Saccharose is found to be the last sugar to disappear on maturation, and the first to appear on germination, but, quoting LEATHES, the intermediate steps are "fascinating in their obscurity." As to the mechanism involved in the transformation from fat to sugar and vice versa, the question arises as to whether it is a process depending upon the metabolism of the living cell and the structure of protoplasm, or merely a reaction or set of reactions catalyzed by plant enzymes which could be performed by the extracted catalysts. Success in discovering the intermediate steps will depend largely upon this. A good bibliography is given.—J. B. RHINE.

Comparative morphology of Poa, Phleum, and Setaria.—NISHIMURA[34] has published the results of a detailed comparative study of these three genera of grasses. Attention has been called[35] to a preliminary notice in reference to *Poa pratensis*. The present full paper deals with the characteristic features of germination, stages in the development of stems and buds, development of the root and its relation to the buds, development of the inflorescence, and polyembryony.—J. M. C.

North American flora.—Part I of volume 33 contains two of the three families of Carduales, Ambrosiaceae and Carduaceae, by RYDBERG, the Vernonieae of the latter family being presented by GLEASON. The Ambrosiaceae include three new genera (*Leuciva*, *Chorisiva*, and *Acanthambrosia*) and 20 new species. The Vernonieae include 4 new species. In this tribe the genus *Vernonia* is presented as including 123 species.—J. M. C.

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[33] TERROINE, EMILE F., État actuelle de nos connaissances sur la formation des graisses au cours de la maturation des graines et fruits oléagineux et sur l'utilization des graisses au cours de la germination. Ann. Sci. Nat. 10. 2^6:1–63. 1920.

[34] NISHIMURA, M., Comparative morphology and development of *Poa pratensis*, *Phleum pratense*, and *Setaria italica*. Japanese Jour. Bot. 1:55–85. *pls.* 4–7. 1922.

[35] BOT. GAZ. 75:109. 1922.

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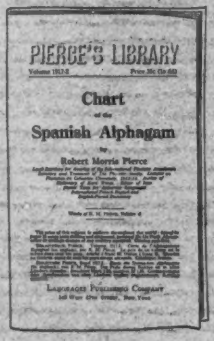

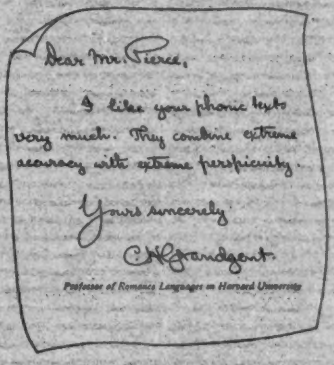
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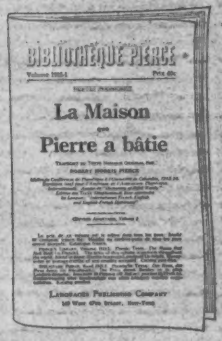

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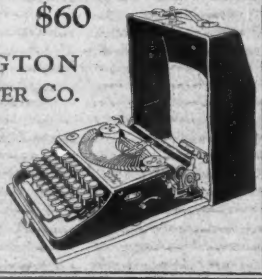

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BUFFALO, N.Y.